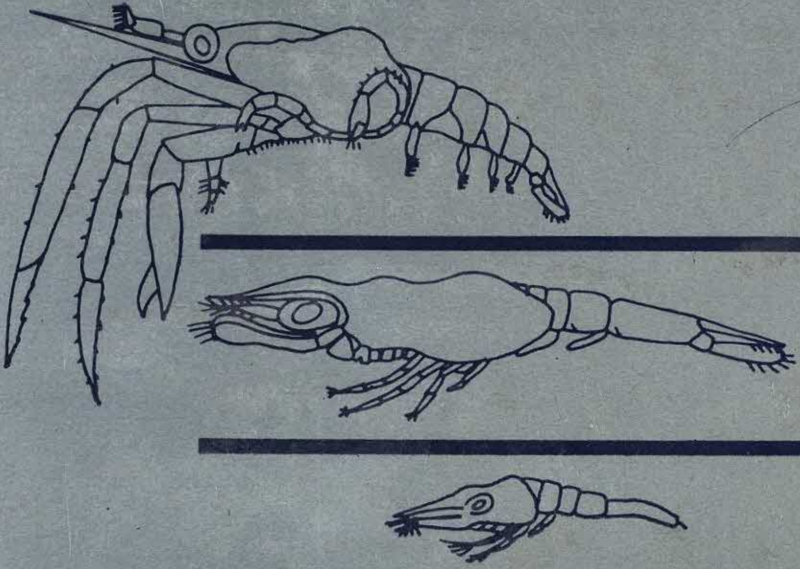

INVERTEBRATE EMBRYOLOGY

R. Nagabhushanam
R. Sarojini

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The knowledge of embryology is essential for every student of zoology. In the university curricula, great deal of emphasis is made in the study of vertebrate embryology while the field on invertebrate embryology is neglected. In fact, there have been practically no textbooks written in the last thirty years dealing with embryology of invertebrates. This textbook on Invertebrate Embryology will be of tremendous help to the students of zoology to fill the lacunae in their knowledge on embryology.

The subject matter is presented in twelve chapters starting from sponges and covering everything up to echinoderms. For the major phyla, the descriptive embryology of the type normally dealt in the regular study is given and then compared with the development of other important animals. Whenever possible, illustrations are given to show the development changes. Besides, some experimental details are mentioned in some chapters dealing with planarians, sea urchins and crustaceans.

Rs. 55.50

Invertebrate Embryology

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Foreword

Large number of books and manuals have been written and are being published on Vertebrate Embryology. But, to the best of my knowledge, during the last three decades or more no book on the embryology of non-chordates has been written either in English or in any other modern Indian language. Similarly, while considerable information is available on mechanisms involved in the functioning of living system, there is very little reliable information on cellular differentiation during development of an organism. One of the reasons for this is ignorance, and lack of information on the processes involved in the development especially of the non-chordates. It is reasonable to assume that fundamental processes that regulate development are less complicated and thus easy to investigate and understand in lower forms, than in higher forms. Obviously, therefore, better insight would be gained if embryology is taught, beginning not at higher forms but of lower forms in which the processes would still be more easily followed and understood. Unfortunately, the tendency among teachers all over the world is to begin either with the frog or rabbit-animals which are quite advanced in the scheme of evolution. Probably easy availability of the material and also of the information on their development may be responsible for their popularity. But for a country like ours where there is no dearth of animals and facilities for maintaining rodents, amphibians and chicks in laboratories are generally not available, it would be wise to develop our own animal models for teaching and understanding development of animals. That we have so far not started serious studies could be due to the fact that there is either lack of information or when present, it is not easily accessible to students and teachers. The present book, it is hoped, will provide to students, teachers and research workers, probably for the first time, information in a book form on descriptive embryology of invertebrates. It is to the credit of the authors that they have tried to include as far as possible information available in Indian animals.

The book, I hope while fulfilling our need for information on the development of non-chordates, will also generate interest among teachers and students to study our own fauna, which is so far has been neglected. The book would be of help both to undergraduate students interested in knowing the facts and also to post-graduate students as a source of information on non-chordate embryology.

Shillong PROF. J.P. THAPLIYAL

Preface

Embryology is a branch of learning which, starting with the embryo and following it through its larval stages to adulthood, inquires into the developmental processes of organs and body systems. While there are several textbooks available to the students of Comparative Embryology of vertebrates in the world, there are hardly any books published in the English language after McBride's Text Book of Invertebrate Embryology (1914) dealing with the whole range of Invertebrate Embryology. The present book was planned to fulfil this lacunae in the field of Invertebrate Embryology.

The subject matter is presented in twelve chapters starting from sponges and covering everything up to echinoderms. For the major phyla, the descriptive embryology of the type normally dealt in the regular study is given and then compared with the development of other important animals. Whenever possible, illustrations are given to show the developmental changes. Besides, some experimental details are mentioned in some chapters dealing with planarians, sea urchins and crustaceans.

In writing this book, we have received assistance and encouragement from many sources. Above all, our gratitude goes to our graduate students who have given practical help in innumerable ways. The present work has been supported by a generous grant from the University Grants Commission under Text Book Project scheme. We are also extremely grateful to Prof. J.P. Thapliyal, Banaras Hindu University, Varanasi and Prof. K. Hanumantha Rao, Andhra University, Waltair for their advise and trenchant criticism. Finally we wish to thank the authorities of Marathwada University for providing facilities in the preparation of the manuscript.

University Campus
Aurangabad

R. NAGABHUSHANAM
R. SAROJINI

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1. *Porifera*

INTRODUCTION

Sponges are animals with the simplest type of body organization next to protozoa. Yet their developmental processes are not in the least as simple. Even though the sponge larvae organization belongs to the low order, the development of each part involves many intricate problems which are not present in the development of other animals.

Regarding the development of the fertilized egg, it is difficult to establish definite demarcations between germinal layers. This is due to the lack of differences among the blastomeres. The egg itself has no definite shape, no membranes, and moves among the somatic tissues by amoeboid movement and can be distinguished only by its larger size.

The adults ponges possess archaeocytes among the mesoglea. These cells are capable of differentiating themselves into any type of cells. Higher animals are said to have some kind of these cells but these come into function only when there is any injury or loss of some part.

H.U. Wilson (1907) squeezed the sponges through bolting silk so that they completely separated into individual cells. These cells were shown to reaggregate and regenerate into a complete sponge. Only epidermal cells, or only choanocytes do not form a perfect animal, but archeocytes are needed to form a perfect animal.

REPRODUCTION OF SPONGES

Sponges reproduce both by sexual and asexual modes. The latter is generally by bud formation. External formation of the bud is called budding, whereas internal formation of the bud is known as gemmule formation. Sexual reproduction is accomplished by the formation of eggs and sperms.

1) **Asexual Reproduction**

In all freshwater and some marine sponges, asexual reproductive bodies, known as gemmules, are regularly formed as part of the life cycle. The gemmules of freshwater sponges form into groups by the gathering of amoebocytes or archaeocytes. Special nurse cells called trophoblasts help in loading the amoebocytes with food reserves which are in the form of glycoproteins or lipoproteins. Other amoebocytes surround the cell mass and arrange into a columnar layer. This columnar layer secretes a hard inner membrane and later a thin outer one. Meanwhile the scleroblasts throughout the sponge body start secreting amphidisc spicules and these are brought into the columnar layer and arranged radially in between the outer and inner membranes. When the gemmule is formed the columnar cells, trophoblasts and the scleroblasts move away, leaving the central mass of archaeocytes. A fully formed gemmule will have a central mass of food-laden archeocytes surrounded by a wall composed of two membranes. In between these two membranes is a layer of amphidiscs. An opening, the micropyle, is present through all these layers.

Freshwater sponges belonging to the family spongillidae produce a large number of gemmules in the autumn and then they disintegrate. Some of the gemmules remain in remnants of the sponge body while the others fall to the bottom. The gemmules can withstand freezing and to a certain extent drying and under favourable conditions, usually in spring, hatch out. According to Zeuthen (1939) the gemmules hatch in about three days at a water temperature of 13 to 21°C. Upon hatching, the cells contained in the gemmule escape from the micropyle and develop into a young sponge by differentiation and rearrangement. The large multinucleate archaeocytes divide into uninucleate ones. Some of those which emerge out first arrange to form the epidermis and the remaining differentiate into choanocytes and porocytes. The spicules are secreted by scleroblasts, which are modified archeocytes. In about a week after hatching a small complete sponge surrounds the empty gemmule shell. Many of the uninucleate archaeocytes, remain undifferentiated, serving as phagocytic amoebocytes or later forming germ cells.

Gemmule formation in the marine sponges was studied by Wilson (1890) in a species of *Tedania*. The gemmule begins as an aggregation of archaeocytes, which become enclosed by a thin layer of flat cells. The surface cells of the mass become columnar and flagellated except at the posterior end. The gemmule then escapes as a flagellated larva. It swims for some time, settles near the nonflagellated pole and loses the flagella. The body cells become differentiated along various lines. These larvae may entirely resemble those arising from fertilized eggs and develop like the latter.

2) Sexual Reproduction

All sponges reproduce sexually by means of typical ova and spermatozoa, but the details are not clearly studied. Regarding the origin of the germ cells there are several opinions. According to the classical account, the cells come only from archaeocytes. Several authors maintain that the sex cells originate from choanocytes (Fig 1.1) while others have described them as arising from the amoebocytes within the mesoglea. The oogonia, an egg mother cell, is first noticed as an enlarged amoebocyte with a large nucleus and conspicuous nucleolus. During the growth period, it acquires reserve food material by engulfing or fusing other similar amoebocytes or through specialized nurse cells (Fig 1.1). Upon attaining full size it undergoes the usual meiotic divisions.

Spermatogenesis has been observed in only a few sponges and in many cases appears to be a rare or sporadic process. In some sponges the sperm mother cell or spermatogonium is an enlarged amoebocyte surrounded by one or more layers of flattened cells. These cells might have been derived by the division of the mother cell or modified amoebocytes. This entire structure is called the *spermatocyst*. The enclosed spermatogonium undergoes two or three divisions giving rise to spermatocytes, which finally give rise to sperms. According to Gatenby (1919) the spermatogonia are transformed choanocytes, and the cells of an entire flagellated chamber may change into sperm cells. The sperms enter other sponges by way of the water current and the

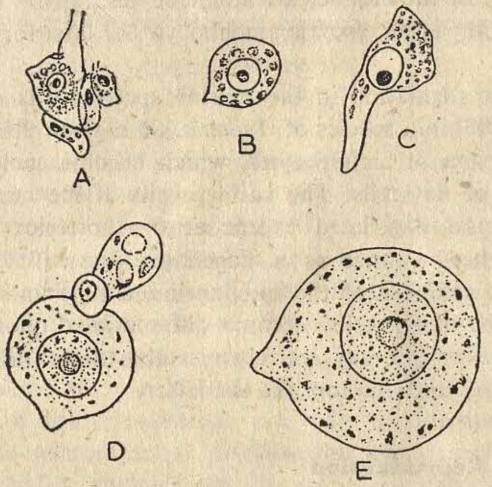

Fig. 1.1. Formation of egg from collar-cell in the sponge, *Grantia*.
A—Transformation of collar-cell into oogonium.
B—Oogonium.
C—Amoeboid stage of oogonium.
D—Oocyte, developed from oogonium, receiving trophocyte caught by nurse cell.
E—Mature oocyte.

eggs are fertilized *in situ.* According to Gatenby, in the group Calcarea, the spermatozoan first enters a choanocyte and is transferred by it to the egg. Choanocytes of this type also serve as nurse cells. Tuzet (1937) reported a similar process in *Cliona* and *Reniera*, where the sperm enters an amoebocyte, which then transfers it to the egg. It is possible that this remarkable kind of fertilization is general for sponges.

The fertilized egg undergoes equal or unequal holoblastic cleavage and develops into a blastula *in situ.* The epidermal cells become flagellated. With the help of these cells the blastula works its way through the parent tissue into the excurrent canals and emerges out through the osculum. After swimming for a few to many hours, the larva attaches to some object and in several hours develops into a small sponge typical of the species.

According to Marshall (1884), the freshwater sponge, *Spongilla laceestris* shows alternation of generation. The animals which develop from gemmules produce reproductive cells; these unite and give rise to sponges which in turn form gemmules.

Although some sponges are dioecious, majority seem to be hermaphrodite. In hermaphrodite sponges, eggs and sperms mature at different times; sometimes they are produced at different levels, i.e., the eggs basally and the sperms apically. Shallow-water sponges usually breed at definite seasons of the year, while the deep-water forms probably breed the year round (Hyman, 1940).

DEVELOPMENT OF REPRESENTATIVE SPECIES

1) Calcarea

Gemmule formation is not found in the group but reduction bodies may arise under adverse conditions. Budding is very common. In some forms, buds remain attached to the parent and this is called continuous budding, while in others the buds eventually become detached and develop into new individuals and this process is called discontinuous budding. Sexual reproduction is by the ova and spermatozoa. The ova is said to come from amoebocytes of the archaeocytic type. In *Sycon* and *Grantia*, where the development is adequately studied, the first recognizable female germ cells are oogonia. These pass into the cavities of the radial canals where they undergo two mitosis, each producing four oocytes. These migrate by amoeboid movement back into the mesoglea, where they increase greatly in size by the aid of special trophocytes, which appear to be transformed choanocytes. One such trophocyte attaches to the surface of each ovocyte and eventually fuses with it.

Poljaeff (1882) studied the spermatogenesis of a species of *Sycon*. He found that the nucleus of the spermatogonium divides into two. One of the nuclei moves towards the periphery of the cell, while the other remains at the centre of the cell. The nucleus at the periphery with a little bit of cytoplasm separates from the main part of the spermatogonium to form a cover cell. The cover cells remain undivided whereas the main spermatogonium divides to form a mass of spermatocytes. The spermatocytes undergo the mitotic divisions, each forming four to six spermatids which transform into spermatozoa. These spermatozoa become covered by an envelope formed by cover cell and this structure is called the sperm ball.

In fertilization, the sperm enters a choanocyte which is adjacent to a ripe oocyte. This choanocyte loses its collar and flagellum and becomes amoeboid. The modified choanocyte containing the sperm then attaches itself to the surface of the oocyte, which forms a conical depression to receive it. The sperm in the meantime loses its tail and its swollen head becomes surrounded by a capsule. The capsule carrying the sperm head penetrates into the oocyte, and the sperm carrying choanocyte departs.

The fertilized egg of *Leucosolenia variabilis* undergoes the maturation divisions and cleaves holoblastically. At the 16-celled stage the embryo lies in the form of a flattened disc under the maternal choanocytic layer. The tier of eight cells next to the maternal choanocyte layer gives rise to the future choanocytes. The latter divide rapidly resembling the micromeres of other metazoan embryos, acquire flagella and face the blastocoel. The future epidermal cells remain undivided for sometime, forming a group of eight cells which resemble the macromere in other metazoan embryos. In the centre of this group arises an opening which functions as a mouth and ingests adjacent maternal cells. This stage is called as the *stomoblastula* by Duboseg and Tuzet (1937). Now occurs a process called inversion, in which the embryo turns inside out through the mouth, so that the flagellated ends of the small blastomeres (micromeres) are on the outside. The embryo is now the typical hollow larva of the Calcarea, called an *amphiblastula* (Fig. 1.2). The greater part of the surface of this larva is covered by small narrow flagellated cells, and the remainder, usually considered the posterior pole, consists of the group of large, rounded, granular nonflagellated macromeres. These nonflagellated macromeres multiply until they come to constitute the greater part of the larval body. The larva is surrounded by a layer of trophoblasts which supply it with nutrition (Fig. 1.3). When completed, the amphiblastula forces its way into the adjacent radial canal and is carried to the exterior. When the larva is swimming, the flagellated hemisphere is directed forward and also usually bears the polar bodies.

Gastrulation takes place when the larva is swimming. The

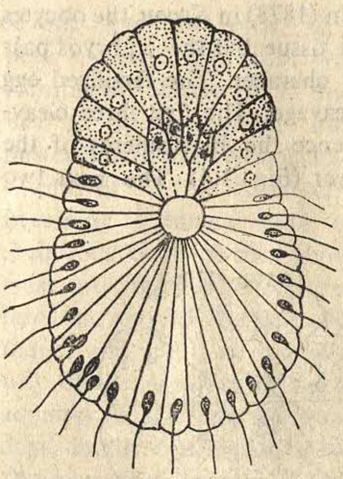

Fig. 1.2. Amphiblastula of *Leucosolenia.*

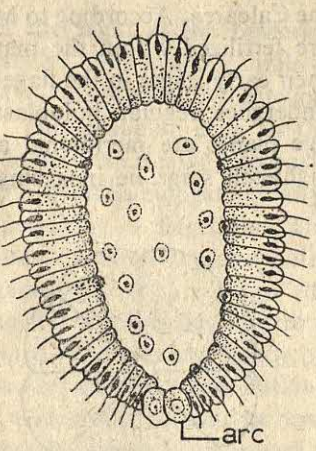

Fig. 1.3. Development of *Leucandra.* Swimming larva showing archaeocytes.

flagellated cell layer invaginates forming an inner mass of cells. Some of the flagellated cells lose their flagella, become amoeboid and fill the blastocoel. This solid type of larva is called the *stereogastrula* or *parenchymula.* The flagellated cells become spherical, with inconspicuous flagella and crowd together in the blastocoel. Dispersed in these cells are found amoebocytes which later change into porocytes. These form cell masses here and there inside the larva and an irregular cavity appears between them which later develops into the spongocoel (paragastral cavity). Some of the epidermal cells migrate to the interior and become scleroblasts. Except for its apical part, the entire paragastral cavity is lined with choanocytes having collar and flagellum. The apical part is the future osculum of the adult. The larva constructed from these tissues gradually become tubular and develops into a small sponge. Each of the masses of porocytes which lie near the surface forms a canal, the animal thus acquiring numerous small incurrent pores. The osculum opens to the outside at the apical end, the choanocytes lining the paragastral cavity become functional and ingest food and the young sponge grows into an adult.

The development of *Sycon rapleanus* is somewhat different from that of *Leucosolenia*, although both the genera belong to the Calcarea. According to Miuchin (1878) in *Sycon*, the oocytes are fertilized within the maternal tissue but the embryos pass their early stages in the flagellated chamber. The fertilized egg undergoes regular holoblastic cleavage. The first three cleavage planes are meridional and hence the blastomeres of the eight-cell stage lie in a single layer (Fig. 1.4). The next two cleavages are equatorial and the embryo at the 32-celled stage acquires a small central cavity. After this the cleavage pattern is irregular leading to the formation of a blastula composed

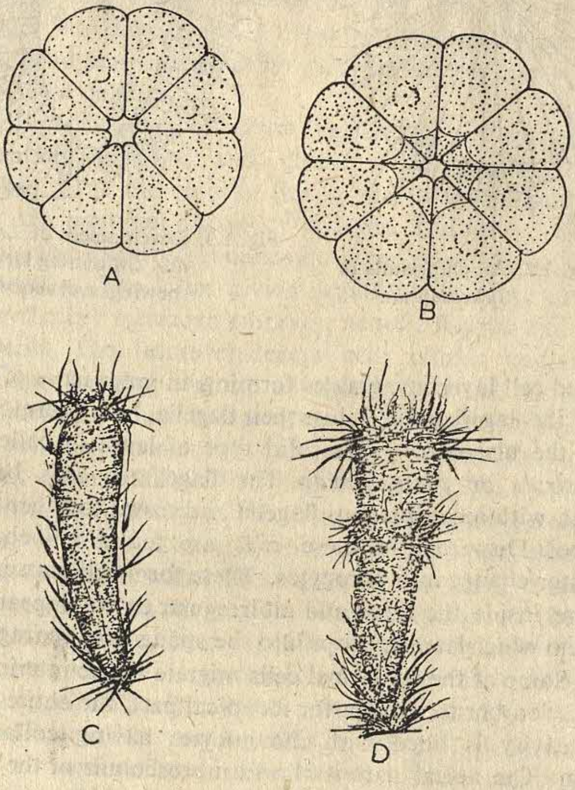

Fig. 1.4. Development of *Sycandra*.
A—8-cell stage.
B—16-cell stage.
C—Ascon type of *Sycandra*.
D—Larva of *Sycandra*.

of one layer of cells, of which the eight at the posterior pole are large and filled with granules. The inside is hollow, forming the blastocoel. In this type of embryo the blastocoel has no opening to the outside. The granular cells at the posterior end are archeocytes. While they are cleaving to form a total of 32, the other cells of the blastula elongate greatly, becoming columnar and each acquiring a flagellum. The archaeocytes divide continuously, and push their way, as a solid mass into the blastocoel (Pseudogastrula). At this stage the larva moves with the help of its flagella, through the maternal tissues and into one of the flagellated chambers and finally by way of the osculum to the outside. It then becomes hollow, acquires bright red pigmentation and swims for one or two days, finally settling on some substratum where it undergoes a striking transformation. The flagellated cells which have made up the anterior part of the larva shift to the inside of the posterior part, hence the anterior region becomes flattened and the blastocoel is compressed to a narrow slit-like space (Fig. 1.4).

After becoming attached to a substratum, the animal elongates into a closed cylinder, composed of an outer layer of flattened cells and an inner layer of flagellated cells. The gelatinous mesoglea develops in between these two layers. Some of the flattened cells of the outer layer migrate into the space between the outer and gastral layers and become porocytes as each cell is pierced by a central canal, while others become scleroblasts within the mesoglea. In this manner the young sponge with a simple canal system of the asconoid type develops into a highly complicated system of flagellated chambers (Fig. 1.4).

2) **Hexactinellida**

The Hexactinellida being deep water forms, have been studied entirely from preserved specimens collected in deep-sea dredging expeditions, and consequently little is known of their reproduction.

Some hexactinellids reproduce asexually by giving off small buds from almost any part of the surface. The grouping of archaeocytes into groups is of common occurrence throughout the order and is interpreted to represent stages in gemmule formation.

The sexual reproduction is poorly known. The most complete account is given by Okado (1928) for the sponge *Farrea sollasii*. In this glass sponge, the eggs are formed within a mass of archaeocytes. The egg cell is larger than the somatic cells, and has an alveolar nucleus. The mature egg is 0.7 mm in diameter, with numerous yolk granules packed around its large alveolar nucleus, and lipid containing vacuoles in the peripheral cytoplasm (Fig. 1.5). The spermatogonia are also differentiated archaeocytes. The spermatozoan has a spherical head and a slender tail. Fertilization appears to take place within the maternal tissues. Cleavage is total and more or less unequal, and the resulting blastula is of the parenchymula type. This embryo develops within the maternal tissue. At first it is planula like, spherical and later become oval. It consists of a central mass of mesoglea containing amoeboid cells. This mass is surrounded by a layer of cells containing yolk granules. The central amoeboid cells arrange themselves in a layer surrounding a central cavity and then change their form to become the future choanocytes. Tetract spicules with long rays appear at the surface and discohexasters form in the interior. At this stage, the larva probably becomes flagellated, escapes from the parent and settles down (Fig. 1.6).

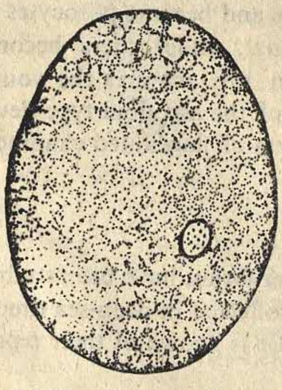

Fig. 1.5. Development of hexactinellid sponge, *Farrea*, egg in body of adult sponge.

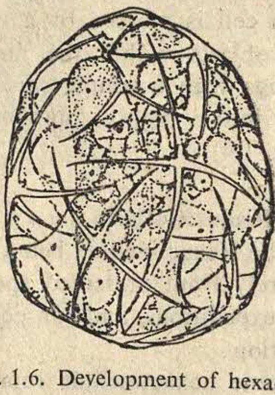

Fig. 1.6. Development of hexactinellid sponge *Farrea*, young sponge, escaped to outside is about to attach.

3) **Tetractinellida**

Asexual reproduction is by external as well as internal budding. The external type of budding occurs in the genus *Donatia*. The bud first appears as a small external protuberance, then it develops spicules and becomes detached from the parent animal (Fig. 1.7), attaches to some object and further develops into an adult. Internal budding is found in the genera, *Fientina* and *Oliona*. This is similar to gemmule formation of fresh-water sponges in certain respects. They differ from the gemmules in not having spicules, inner membrane and micropyle.

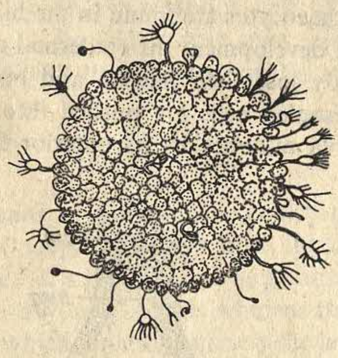

Fig. 1.7. External budding in tetractinellid sponge, *Donatia*.

Sexual reproduction was studied in the genus *Plakina* (Fig. 1.8) by Mass (1909). The cleavage is equal, holoblastic and the

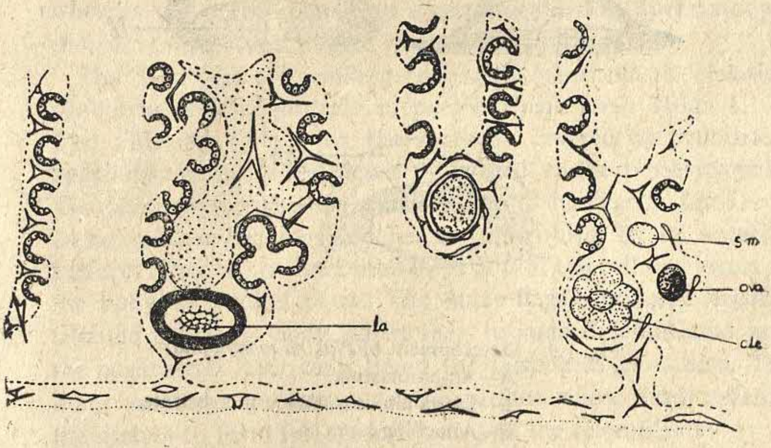

Fig. 1.8. Part of tetractinellid sponge *Plakina*.
cle—embryo at cleavage stage.
la—larva.
ova—ovum.
sm—sperm ball.

resulting blastula is ovoid to pear-shaped. Flagellated cells cover the outer surface of the blastula, a few granule filled archaeocytes are found in the blastocoel. During the course of the development the epidermal cells at the posterior end of the body lose their flagella and become filled with granules. The mesoglea cells are pushed into the blastocoel (Fig. 1.9). The larva attaches by its anterior flagellated half which becomes

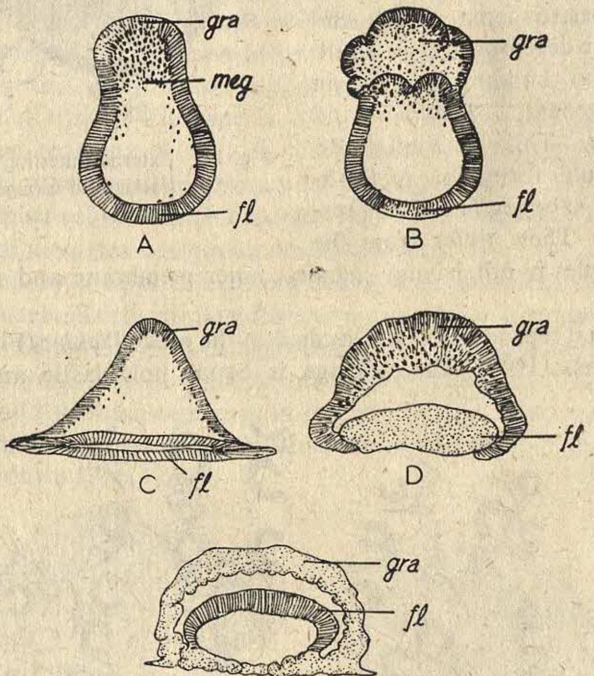

Fig. 1.9. Development of *Plakina monolopha*.
A—Amphiblastula.
B—Larva about to attach to substrate.
C—Attaching larva.
D—Larva in late attachment stage.
E—Rhagon stage.
fl—flagellated cells.
gra—granular cells.
meg—mesoglea cell.

flattened and then invaginates inwards, so that the flagellated cell layer becomes surrounded by the granule containing columnar epithelial layer making up the posterior half of the body (Fig. 1.9), a blastopore is formed and it soon closes down by the surrounding epithelial cells. The larva at this stage is called the rhagon.

4) **Keratosa**

Asexual reproduction by means of gemmules is common in this group. The gemmules of Spongillidae differ from those of marine sponges in being enclosed in a hard shell. The gemmules of Spongillidae hatch out into a young sponge, whereas those of marine sponges into a larva which resembles closely the larva arising from a fertilized egg.

Many of the keratose sponges are dioecious whereas the hermaphrodite ones are protandrous. The eggs as well as the sperms are derived from archaeocytes. The egg has a large nucleus and the cytoplasm is granular. It is enclosed by a trophic membrane which is probably derived from the parenchymatous tissue near the egg. The spermatogonium divides into two cells, one of which develops into an envelope cell, whereas the other becomes a spermatocyte. The spermatocyte divides several times to give rise to spermatozoa.

The fertilized egg undergoes unequal holoblastic cleavage producing a *stereogastrula* or *parenchymula* larva (Fig. 1.10). The anterior surface of the larva is covered by cylindrical flagellated cells. The skeleton is formed at the posterior pole. The parenchymula swims with the help of its flagella for about 24 hours, then becomes attached to some object by its anterior surface. Then a process called inversion of the cellular layers of the body wall takes place. The outer flagellated cells become situated inside the body, where they become differentiated into the paragastral layer cells, lining the flagellated chambers. The inner amoebocytes emerge to the surface where they arrange themselves to form the dermal layer and the scleroblasts.

In freshwater sponges Breen and Meewis (1938) have found that the fertilized egg cleaves to form a stereoblastula composed of three sizes of cells, the micromeres, macromeres and the mesomeres. The micromeres which are at the anterior pole spread over the embryo by a process called epiboly and multiply

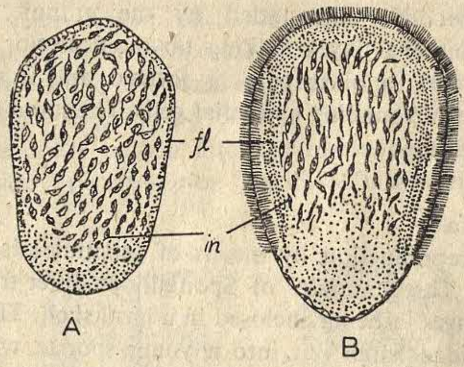

Fig. 1.10. Development of marine monaxonid sponge, *Esperella*.
A—Parenchymula larva containing cell mass in its interior.
B—Swimming larva.
fl—Flagellated cell layer.
fl.c—flagellar chamber.
in—inner cell mass.

rapidly so as to form a surface layer of small cells that become flagellated while the embryo is still in the maternal tissue. The cells of the inner mass transform into scleroblasts, choanocytes and amoebocytes. The scleroblasts and the choanocytes are derived from macromeres. The embryo escapes as a completely flagellated larva and after swimming for a few hours to several days, it fixes by the anterior pole and the canal system develops from the cavity already present.

2. *Coelenterata: Cnidaria*

GENERAL DEVELOPMENT

Asexual reproduction takes place continuously in the coelenterata, but sexual reproduction occurs only once in a year. Most of them breed sexually between early spring and autumn, some of them reproduce during winter, whereas some tropical species may breed several times a year. In the free-swimming medusa-type of forms, the eggs are liberated into the surrounding water and fertilization takes place either when they are suspended in the sea-water or when they sink to the bottom. In the sessile, polyp type of forms, the eggs are fertilized when they are still attached to the parent tissue. If the fertilization takes when the eggs are in a suspended stage, the embryo passes through a free-swimming *Planula* stage before settling to the bottom and transforming to a polyp-form. This polyp buds off medusae which in their turn develop reproductive organs and reproduce sexually. In the group Actinozoa, no medusa stage ever appears, the polyp-stage alone gives rise to planulae which attach and become polyps, producing reproductive cells which grow into planulae. In the Hydrozoa, the reproductive cells are ectodermal in nature, while in all other coelenterata, including ctenophora, they are endodermal in their origin.

The cleavage in the group Cnidaria is said to be radially symmetrical, whereas in Ctenophora it is bilaterally symmetrical. In the genus *Spirocodon*, the first cleavage furrow does not reach the vegetal pole, so there is practically no cleavage activity at that pole (Fig. 2.1). As the cleavage advances the blastula is formed. This may be either a coeloblastula or a stereoblastula. The blastula advances to become the gastrula. The endoderm of gastrula in many cases is formed by invagination.

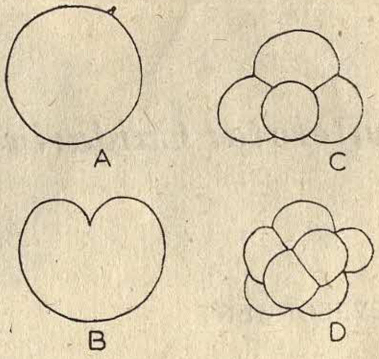

Fig. 2.1. Cleavage in the medusa *Spirocodon*.
A—Fertilized egg.
B—Successive stage of first cleavage.
C—4-cell stage.
D—8-cell stage.

Invagination begins at the vegetal pole of the blastula by proliferation of cells, and these cells accumulate on the inner side of the blastular wall. In polarization, the cells of a limited area at the vegetal pole proliferate into the blastocoel. Delamination is a special mode of gastrulation peculiar to the coelenterates, in which all the cells of the blastula divide into the blastocoel. In this way the outer and inner body layers, the ectoderm and endoderm, are formed. Later, the mesoglea is secreted between these two layers.

The eggs of many Actinozoa and Hydrozoa are opaque, containing large amounts of yolk. In these cases it is difficult to make accurate observations of the cleavage patterns. The cleavage appears to be superficial. In some cases the blastomeres separate into small ectodermal and large endodermal cells. In some forms, a gastrula is formed where the outer layer becomes the ectoderm and the inner layer, the endoderm. In such cases, the yolk remains in the blastocoel and gradually absorbed.

As the germ cells are being formed, the ectodermal cells develop cilia, sensory and gland cells get differentiated, and a free swimming planula larva is produced. The planula larvae of Stauromedusae do not possess cilia and hence creep at the bottom. In Trachymedusae, Narcomedusae and Siphonophora the planula larvae are solid without a blastocoel, whereas in others there is a blastocoel.

In many cases, the planula larva becomes attached and develops into a polyp. In Zoantharia and Ceriantharia the planula larva while still swimming, transforms into a temporary swimming polyp form, and is called by special larval names. The siphonophores, *Porpita* and *Valella* also develop into a special type of larva. All these larvae undergo metamorphosis to become adult forms. In Scyphomedusae after the planula has developed into a polyp, it produces a special *Strobila stage*, which asexually gives rise to the young larval medusae (ephyra). This ephyra passes through metamorphosis to become the adult medusa.

HYDROZOA

Among the Hydrozoa, each species has a polyp and medusa stage. These two stages appear alternately to give a regular alternation of generations. The extent of development of each stage depends upon the particular species. In some forms the polyp stage may be predominant, while the degenerate medusa does not separate and remains attached to the polyp in the form of a sporosac. In some species, the whole life cycle may be pelagic, only the medusa is well developed, while the polyp is present in a modified or rudimentary state. In the Hydrozoa only the group Hydroidea has a conspicuous polyp stage.

DEVELOPMENT OF *TUBULARIA MESENBRYANTHEMUM*

In the development of *Tubularia* there is no medusa stage and retains only the polyp form. It lives in shallow water on rocks and loose stones along the sea-shore or attached to eel grass. The polyp reaches to a height of 3.8 cm. It is generally found on all parts of the European and American shores.

The gonophores which are present on the polyp produce reproductive cells continuously by budding. This type of gonophore is considered to be a degenerate medusa called as the sporosac, which does not get detached from the polyp. The gonophores are formed in the space between the two circlets of tentacles of the hydranth. A number of processes appear at the base of the hydranth and at the end of each one of these processes is formed a gonophore, so that the entire structure resembles a bunch of grapes. The species of *Tubularia* are dioecious, the gonophores of a colony are either all male or all female and often exhibit sex differentiation to a certain degree. A manubrium is present at the centre of each gonophore. In the case of male gonophores, sperm sacs and in the case of females several eggs are given out from this manubrium. The eggs are fertilized *in situ* by the spermatozoa of the male gonophores.

The eggs of *Tubularia* contain a lot of yolk, hence the cleavage is rather irregular. Since the number of eggs in a single gonophore are more, this also tends to make the cleavage more irregular. The cleavages are somewhat polynucleate, the cytoplasm accumulates at the surface and the yolk towards the centre and this gives the effect of a superficial cleavage. The inner endodermal cells are a bit irregular in the beginning, but eventually form a definite layer. With this type of cleavage, the division of the blastomeres and the germ layer formation takes place simultaneously.

If only the cleavage is observed, it is found that at the 16-celled stage of the irregular cleavage, a small space is formed at the centre of the blastomeres. This stage is called as the blastula stage. As the cleavage proceeds the small blastocoel disappears and the embryo becomes a solid morula. At the time of the formation of the morula, the cells of a part of the blastula wall divide rapidly many times and these cells proliferate into the blastocoel. The cells of the original blastula become the ectoderm and the proliferated cells become the endoderm.

Small empty spaces make their appearance at the centre of the solid morula. These spaces eventually join at the centre, giving rise to a single cavity which later becomes the adult coelenteron. At this stage the embryo becomes flattened and plate-like. A number of small processes which are the rudiments of aboral tentacles, develop. As the development proceeds these processes bend towards the concave side of the embryo and increase in length. Meanwhile at the opposite convex side the ectoderm and the endoderm become very thin and eventually a mouth opening makes its appearance. At this stage a tendency to bend toward the aboral side becomes pronounced (Fig. 2.2).

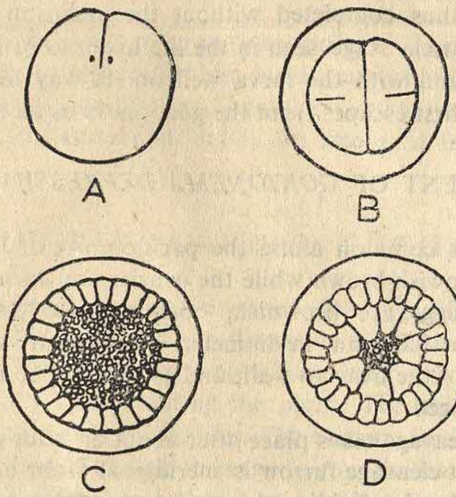

Fig. 2.2. Development of medusa, *Gonionema*.
A—First cleavage.
B—Beginning of third cleavage.
C—Blastula.
D—Formation of endoderm.

Around the mouth opening at its upper end a number of small processes are formed which are the rudiments of oral tentacles. In the meantime, the aboral tentacles, become long and slender and the aboral end of the body itself grows to about the same length as that of the tentacles. The larva at this stage is called the *actinula* larva.

Upto the formation of the actinula stage, development takes place inside the gonophore and the actinula larva escape to the outside through the mouth of the gonophore. When once they are free, they sink to the bottom, where they move about with the help of the oral and aboral tentacles and finally attach themselves to rocks or any other suitable substratum by their aboral ends. After attachment, the actinula increases in height, the part bearing the oral and aboral tentacles becomes the hydranth. The stalk secretes a chitinous perisarc and this modified actinula is called the *hydrapolyp*. From the attached base a number of stolons are put out and these in turn give rise to new polyps, forming a colony. The gonophores appear for the first time during the breeding season. The development of *Tubularia* is thus completed without the inclusion of a free-swimming planula stage seen in the life histories of most other coelenterates and with the larva well on its way to the adult state when it first escapes from the gonophore as an actinula.

DEVELOPMENT OF *GONIONEMA DEPRESSUM*

The species is common along the pacific coast of Japan. The testes are yellowish brown while the ovaries are dark brown in colour. The eggs are brownish, opaque, with less yolk and measure about 0.07 mm in diameter. The eggs are released by the breaking of the ovarian wall, and as they fall to the bottom they are fertilized.

The first cleavage takes place after about an hour of fertilization. The first cleavage furrow is meridional from animal pole to the vegetal pole, dividing the egg into two blastomeres. The second cleavage starts at the animal pole but at right angles to the first and takes about 50 minutes to complete. The third cleavage is equatorial giving rise to upper four and lower four blastomeres. The upper four blastomeres are turned by 45° and wedged between the four lower blastomeres. This resembles the spiral cleavage, but the latter divisions fail to show the characteristics of the mode of cleavage.

The endoderm is formed by delamination. The ectodermal cells acquire cilia and the larva takes the form of a planula which starts rotating inside the chorion. After about an hour, the chorion breaks and the larva is set free. When once the larva is outside it becomes elongated, swims for several days and then settles to the bottom. The larva then loses the cilia, creeps about for a while before changing into a polyp.

The polyp first develops a mouth opening and two tentacles appear at the opposite ends of the mouth. At a later stage two more tentacles appear at right angles to the first pair. Nothing more is known about the polyp phase of this species but the development of an Adriatic species, *G. vindoborensis* (Fig. 2.2) has been investigated. The number of tentacles of the polyp may increase to five or six, but during the four-tentacled stage, bodies like planulae which lack cilia are budded off (fustules). This budding is repeated several times and the fustules are believed to grow into polyps. The polyps then produce one to several medusa buds, each of which develops radial canals, manubrium, tentacles, statocysts, a velum and other structures and finally set free as a free-swimming medusa.

DEVELOPMENT OF *SPIROCODON SALTATRIX*

This species breeds during the winter. The eggs of this medusa are about 0.06-0.07 mm in diameter. There is no external membrane. The cytoplasm is colourless and highly transparent. They generally spawn during the night. The mitotic divisions are completed within the ovaries. The eggs are fertilized as soon as they are shed. The first cleavage furrow divides the egg into two, starting at the animal pole and reaching the vegetal pole. The cleavage furrow does not cut through at the vegetal pole. The mitotic spindle becomes V-shaped, and the daughter nuclei are drawn downward and towards the furrow. The second cleavage furrow appears about half-an-hour after the first, is perpendicular to the first and produces four blastomeres. The third cleavage is equatorial, the eight blastomeres are tilted at an angle of 45° so that the upper and lower layers are wedged against each other in an alternating way, resembling the arrangement in spiral cleavage (Fig. 2.1).

The consequent cleavages increase the number of cells and finally becomes the blastula. First the blastula is elliptical in shape but gradually becomes egg-shaped. Ten hours after fertilization, the blastula becomes covered with cilia, the anterior end is rounded and the posterior end pointed. The larva at this stage is a swimming planula. It is not known when it changes to the polyp form. However, small medusae with four tentacles and four tentacle swellings make their appearance every January, medusa budding is supposed to take place at that season. As this medusa grows and its tentacles elongate, a pair of new tentacles come up on each side of the old ones. The new tentacles tend to push the older ones upward, making the bell margin eight-lobed. The manubrium acquires four lips, which eventually become folded and filled. Following such a course of development, this medusa may reach a height of 50

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mm, and is one of the largest and most highly differentiated and morphologically complicated of the Anthomedusae.

GENERAL DEVELOPMENT OF HYDROIDEA

The members of the Hydroidea have both a polypoid and medusoid generation. These appear alternately, giving an alternation of generations. In many cases the medusoid generation is very much reduced and becomes attached to the polyp. Various degrees in this reduction of the medusoid generation can be recognized. These are divided into eumedusoid, cryptomedusoid, heteromedusoid, styloid etc. (Fig. 2.3). In some species, the degree of reduction is different between the sexes, the female for instance, separating as a free medusa, while the male is represented by only a sporosac.

The reproductive cells of the Hydrozoa are ectodermal in origin and usually penetrate into the endoderm after completing maturation. The spermatozoa are flagellate. The eggs that are produced in a sporosac are likely to be large and yolky, while those that are formed by medusa tend to be small and have little yolk. Depending upon the amount of yolk, the latter development varies considerably. The eggs with little yolk are shed into the sea water and fertilized there. The cleavage is regular, total and practically equal. The first two cleavage planes are perpendicular to each other while the third is horizontal. From this point the cleavage follows the radial type with the micromeres at the animal pole and macromeres at the vegetal side. The simply organized Hydromedusae are also known to cleave totally but irregularly. Eggs which undergo regular radial cleavage tend to form blastulae with small blastocoels or even with none at all, and in many cases the blastocoel is later completely filled with endoderm. While the alecithal medusa eggs form coeloblastulae, the sporosac eggs of such genera as *Clava, Gonothyrea* and *Plumularia* are likely to develop into stereoblastulae. In the coeloblastula, the endoderm is formed by invagination or polarization. While in stereoblastula it tends to be irregular. In the yolky eggs like those of *Tubularia* and *Enderdium*, the cleavage is irregular, giving rise to multinucleate cells, with the cytoplasm accumulated at the surface and the yolk left in the centre of the embryo. This type looks like a

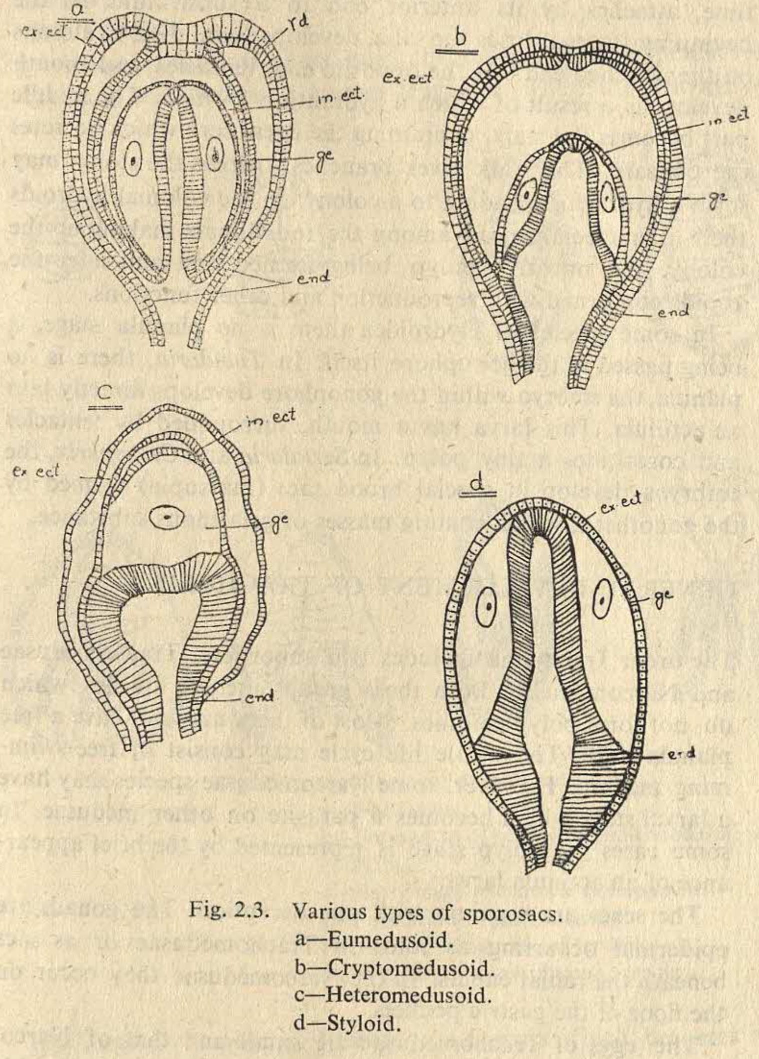


Fig. 2.3. Various types of sporosacs.
a—Eumedusoid.
b—Cryptomedusoid.
c—Heteromedusoid.
d—Styloid.

superficial cleavage, with the ectoderm cells arranged around the surface and few nuclei in the interior where endoderm cells are formed in an irregular manner.

As the coeloblastula proceeds to the gastrula stage it usually swims as a free planula. As it grows, it becomes oval, develops sensory and glandular cells at its anterior end. In many cases, the planula, after leading a free-swimming existence for sometime, attaches by its anterior end to a substratum. At the beginning stages it loses the cilia, develops pedal discs or stolons on the attached end. At the opposite end, tentacles and mouth develop as a result of which a hydranth is formed. The middle part becomes the stalk, containing the coenosarc which secretes the perisarc. The stalk gives branches whereas the stolon may form a hydrorhiza leading to a colony. In the colonial hydroids there is a specialization among the individuals making up the colony, the nutritive polyp being formed first and later the zooids concerned with reproduction and other functions.

In some species of Hydroidea there is no planula stage, it being passed in the gonophore itself. In *Tubularia*, there is no planula, the embryo within the gonophore develops directly into an actinula. This larva has a mouth, surrounded by tentacles and constitutes a tiny polyp. In *Sertularia* and *Orthophrix*, the embryos develop in special brood sacs (marsupia) formed by the gonotheca, which contain masses of gelatinous substance.

GENERAL DEVELOPMENT OF TRACHYLINA

The order Trachylina includes two suborders, Trachomedusae and Narcomedusae. Both these groups include species which do not form polyp colonies. Most of them may not have a free planula stage. The whole life cycle may consist of free-swimming medusa. However, some Narcomedusae species may have a larval stage which becomes a parasite on other medusae. In some cases the polyp stage is represented by the brief appearance of an actinula larva.

The sexes are separate with one exception. The gonads are epidermal occurring as folds in Trachomedusae or as sacs beneath the radial canals. In the Narcomedusae they occur on the floor of the gastric pockets.

The eggs of Trachomedusae are small and that of Narcomedusae are large. The cleavage pattern varies considerably according to the species. The blastula may be a coeloblastula or stereoblastula. In the coelobastula the endoderm formation is by multipolar proliferation and in the stereoblastula it is by delamination method. A planula is formed at the time of gastrula and this followed by the secretion of large amount of mesoglea. Then tentacles and mouth appears and the planula passes into an actinula stage, which gradually metamorphoses into an adult.

Among the Trachymedusae, the mode of development of *Aglaura* and *Liriope* is known (Fig. 2.4) where the cleavage is

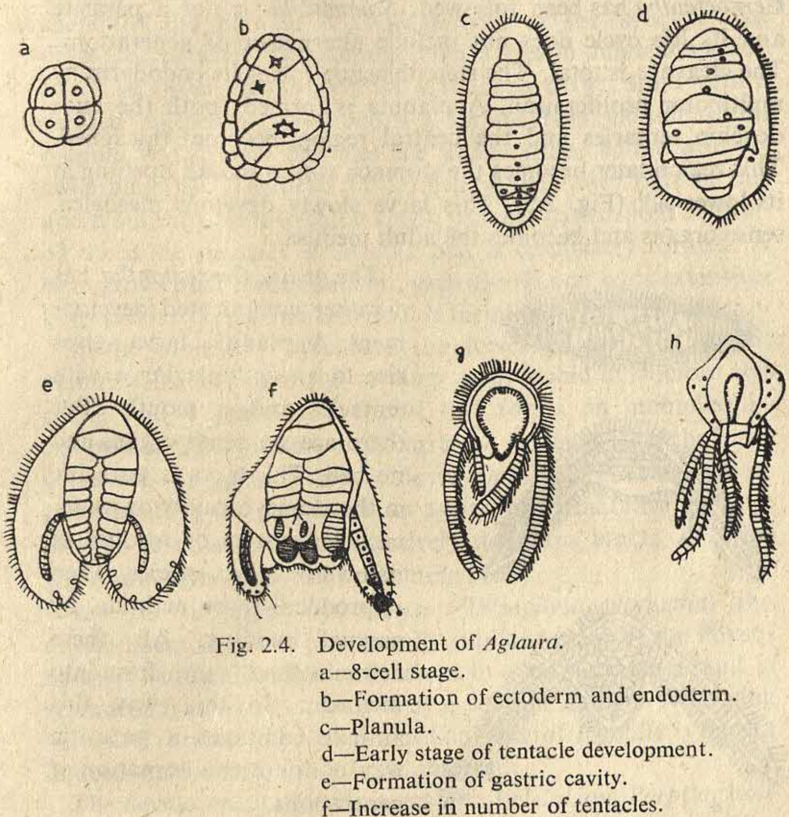


Fig. 2.4. Development of *Aglaura*.
a—8-cell stage.
b—Formation of ectoderm and endoderm.
c—Planula.
d—Early stage of tentacle development.
e—Formation of gastric cavity.
f—Increase in number of tentacles.
g and h—Formation of young medusa.

total, equal or unequal. It leads to a morula which becomes a ciliated planula. Endodermal cells at the posterior end of this larva protrude outward forming the cones of the tentacles. The tentacles increase in number and the cilia are lost as the bell of the medusa takes its shape.

The development of *Liriope* shows few differences. The cleavage is total and equal producing a coeloblastula. The endoderm formation is by delamination. The mesoglea is laid down abruptly between ectoderm and endoderm as there is no planula and actinula stage. This embryo becomes the adult medusa by developing tentacles, velum, manubrium and other organs characteristic of the adult.

Of the Narcomedusae the development process of *Solmundella, Cernoctantha* has been followed. *Solmundella* is not a parasite and its life cycle does not include alternation of generations. The cleavage is total. The stereoblastula forms its endoderm by multipolar proliferation. A planula is formed, both the ends develop tentacles and the central region becomes thickened. This region later becomes the stomach with a mouth opening at its lower side (Fig. 2.5). This larva slowly develops mesoglea, sense organs and becomes the adult medusa.

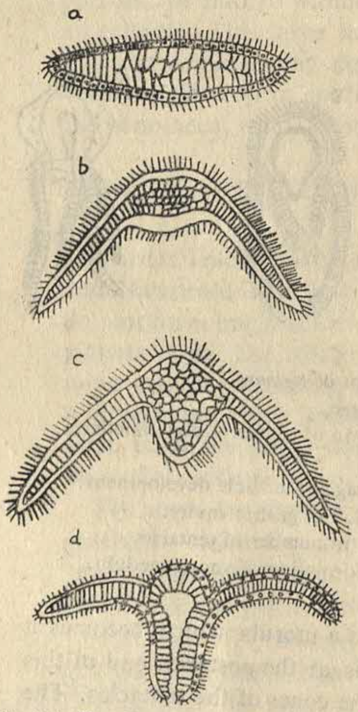


Fig. 2.5. Development of *Solmundella.*

The genus *Cernoctantha* has a rather complicated development. A planula larva gives rise to an actinula larva with tentacles and a mouth. But there are no sense organs and no bell. This larva is parasitic in the stomach cavity or water vascular system of medusa of its own or other species. Then it produces more actinula by asexual budding. All these actinulae finally transform into medusae. In this way this animal combines a parasitic way of life with alternation of generations.

GENERAL DEVELOPMENT OF SIPHONOPHORA

The Siphonophora are pelagic animals forming highly polymorphic colonies by means of budding. The individuals of the colony which are concerned with reproduction are called as gonophores. The gonads of a single colony may be either male or female but the colony as a whole may have either gonophores all of one sex or of both sexes, depending upon the species.

In general the cleavage is total and equal giving rise to a morula. Gastrulation is by delamination. The stereogastrula develops cilia on its outer surface and forms a planula. The shape of the planula is characteristic of the species. The development after the planula stage varies in different species. In the suborder Calyconecta the planula is ovoid, solid with a ciliated surface. A small bud is given out from one side of this planula which later becomes the undersurface of the primary swimming bell. Tentacles are formed on the lower part of this and from the opposite end a single primary gastrozooid is formed when the primary swimming bell is completely formed. A stem grows out between bell and gastrozooid and buds extensively to produce the other individuals forming a large colony.

In the suborder Physonecta the lower or oral end of the planula also becomes the primary gastrozooid but before this change takes place, the aboral end forms an umbrella-like expansion. This primary or larval bract serves to float the larva while the pneumatophore is developing. The pneumatophore arises beneath the primary bract as an ectodermal invagination. Near to this are budded successively definitive bracts, dactylozooids and eventually the swimming bells.

In the suborder Cystonecta (*Rhizophysa*, *Physalia*) the development seems to be generally similar to that of the Physonecta. The pneumatophore develops to a still larger size and is separated from the gastrozooid by a septum and no swimming bells are formed. The early development of *Physalia* is passed in deep water and has not been observed.

The development of such forms like *Velella* and *Porpita* show rather wide divergence. The earliest known larva, termed as *Conaria* is a hollow sphere with an aboral thickening. The hollow sphere represents the primary gastrozooid. The aboral thickening contains a small air sac, a system of eight entodermal canals, a ring-shaped nematocyst and an entodermal plug which projects into the coelenteron of the primary zooid. The *Conaria* larva is said to sink into deep water where it continues to develop and only appears at the surface after reaching the next stage which is called a *Rataria* larva. In this stage, the pneumatophore is greatly enlarged. At the junction of the float and the primary zooid a ring-like budding zone is formed from where new zooids are budded out. The rataria larva metamorphoses and gradually builds up the complex structure of the adult siphonophore.

In several species belonging to the Calyconecta, some of the units called *Cormidia* may separate from the colony and take up independent existence. These are called the *endoxides*, they develop gonads, and carry out sexual reproduction. The fertilized eggs give rise to colonies like the original one.

SCYPHOZOA

In the Scyphozoa in general there are both polypoid and medusoid types, but the polypoid generation is insignificant in comparison with the large conspicuous medusoid stage. These two types appear in turn to establish a general alternation of generations. The Scyphozoa is classified into five orders: (1) Stauromedusae, (2) Cubomedusae, (3) Coronate, (4) Semaeostomae, and (5) Rhizostomae. Of these, the Stauromedusae are sessile and the Cubomedusae are pelagic. These two orders have several characters in common and differ from the other three orders in many morphological and embryological characters.

Morphologically, the Stauromedusae are medusoid in their upper part and polypoid in their lower part. Since they do not give off a free-swimming medusa, there is no asexual reproduction and hence no alternation of generations. The development of the Cubomedusae is known upto the point of the formation of the polyp. How the medusa is given out is not known. In the other three orders the usual alternation of generations is seen.

Sexual maturity commonly occurs in spring or summer. The sexes are separate except in *Chrysaora*, which is somewhat hermaphroditic. The reproductive cells are endodermic in origin, cleavage is total and radial, the eight-cell stage showing some spiralling tendency. The first two cleavages are equal. As the cleavage proceeds, a morula and then a coeloblastula are formed. Formation of the endoderm is by invagination. In some cases polarization and epiboly also occur. The gastrula eventually becomes a planula which leads a free-swimming existence for a while and then attaches and becomes a *Scypho-*

polyp. The scyphopolyp develops a pedal disc, a mouth opening, and two tentacles that become four, eight and finally sixteen. At a later stage, septa and sub-umbrellar funnels are formed. As the scyphopolyp develops one or more lateral grooves are formed. Above each groove, eight pairs of lappets are formed. The tentacles retrogress and eight sensory organs or *rhopalia* are formed between the lappets. The lateral grooves deepen until the ephyra is completely separated as an independent unit. During the free-swimming existence the ephyra larva acquires tentacles on each body radius, the gastrovascular cavity takes a complex structure and finally transforms into an adult with more tentacles and other appendages. An ephyra which has grown to some extent and is about to metamorphose is known as *metephyra*.

DEVELOPMENT OF *THAUMATOSCYPHUS DISTINCTUS*

Thaumatoscyphus is dioecious, the eight pairs of gonads lie along each inter radius. The male and female gametes are shed into the sea where fertilization takes place. The spermatozoa are very small, the heads are small, triangular, whereas the tails measure about 70-80 μ in length. The eggs are pale yellow due to the presence of microscopic yolk granules and measure about 50 μ in diameter. The pronucleus of the egg always lies towards one pole. After fertilization the egg becomes surrounded by two inconspicuous membranes. The first cleavage furrow starts at about two hours after fertilization. It is total and about equal. The second cleavage is perpendicular to the first. At the second cleavage the two blastomeres do not divide simultaneously but there is always a time lapse. The third cleavage is horizontal, a little above the equator. The four upper, smaller blastomeres turn horizontally to a slight extent so that they tend to slip between the four lower blastomeres. Till now the cleavage is radial and practically total and equal. Now it loses its irregularity and the outline of the blastomeres becomes indistinct. The embryo becomes a morula. Instead of becoming a blastula, the interior is filled with cells which are about the same as those of the outer layer (Fig. 2.6). The endoderm formation is by unipolar proliferation. After the endoderm has been formed, the ectoderm cells flatten and become arranged in a layer around the outside of the embryo, while the endoderm cells which are now large and vacuolated line up longitudinally in the interior. At this stage the embryo breaks from the fertilization membrane and becomes a long, slender, worm-like planula (Fig. 2.6). This planula larva differs from the other planulae in not having a blastocoel and cilia. Since there is no ciliation, it cannot swim but creeps at the bottom with a vermiform type of movement. A number of such planula are found collected in masses which are surrounded by a shell made up of sand grains and debris.

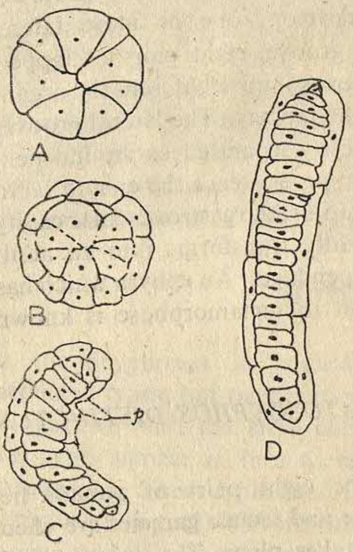


Fig. 2.6. Development of the Scyphomedusa *Thaumatoscyphus distinctus*.
A—16-cell stage.
B—More advanced stage.
C—Planula with ectodermal process (p).
D—Extended planula.

The development of *Thaumatoscyphus* has been observed only to this extent, but information is available about later stages of other Stauromedusae like *Haliclystus*, *Sesakiella* etc. In these forms when the planula finally attaches, it takes on a hemispherical form and before transforming reproduces asexually by budding. The planulae produced in this way grow and form other hemispherical cell masses which finally transform into polyps. The polyp eventually forms a hypostome, pedal disc, tentacles, anchors, infundibulum, gastral filaments etc. New tentacles are subsequently formed in a regular arrangement at each per-radius.

The points in which the development of Stauromedusae differ from the other orders are: (1) there is no blastocoel; (2) the planula is non-ciliated and hence no swimming; (3) locomotion is by vermiform type of movement; (4) the planula changes into a scyphopolyp which in turn becomes a single young medusa; and (5) there is no alternation of generations.

The Cubomedusae have a number of characteristics in common with the Stauromedusae, but the development of Cubomedusae is not very well known. In the case of the genus *Charybdea* the planula and scyphopolyp have been observed. The development in between these stages is not known. Observations on the genus *Charybdea* show that the fertilization takes place in the gastrovascular cavity. The cleavage is total and equal and follows the same pattern as that of the Stauromedusae. The morula gives rise to a Coeloblastula. The endoderm formation is by multipolar proliferation or by delamination. The embryo becomes a gastrula. The gastrula becomes ciliated and transforms into a small pear-shaped planula. After swimming for about two or three days it becomes sessile. It then loses its cilia and becomes a plate-like mass of cells from which two tentacles arise. As the tentacles elongate, the body also increases in length, a small hypostome is formed and two new tentacles make their appearance. This is all that is known about the development of this species at present (Fig. 2.7).

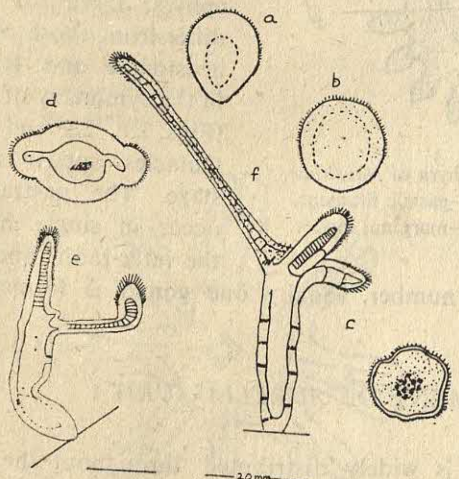


Fig. 2.7. Early development of Scyphomedusa *Charybdea*.
a, b—Successive stages of planula.
c—Attached planula.
d—Formation of two primordial tentacles.
e, f—Formation of Scyphopolyp.

DEVELOPMENT OF *NAUSITHOE PUNCTATA*

Many of these medusae are deep water forms and their embryology is almost completely unknown. The genus *Nausithoe* is a coastal form, and hence a detailed study of its development has been done. The eggs are generally small. The cleavage is radial, equal and total and which eventually gives rise to a morula and blastula. Gastrulation may be either by invagination or by irregular, monopolar proliferation. The gastrula becomes a ciliated planula which detaches itself from the fertilization membranes and starts a free-swimming existence. The planula after sometime attaches by a plate-like cell mass on its underside, loses its cilia and separates into an inner and outer cell layer. The surface becomes covered by a hard transparent layer and later a mouth and tentacles are formed. The polyp is well developed and is commonly known as *Stephenoscyphus*. It grows to a height of 10 cm and starts strobilization. The ephyra larva of *Nausithoe* differ from those of the Semaeostomae and Rhizostomae in the symmetry of its lappets (Figs. 2.8, 2.9 and 2.10). The tentacles appear at a later stage. The gastral filaments occur in single numbers on the inter-radii and gradually increase in number. Finally one gonad is formed on each adradius.

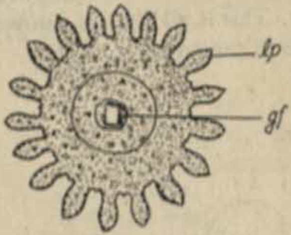


Fig. 2.8. Ephyra of *Nausithoe*.
gf—gastric filament.
lp—marginal lappet.

DEVELOPMENT OF *AURELIA AURITA*

This genus is widely distributed throughout the temperate waters of the Pacific and Atlantic oceans and the Mediterranean Sea. Sexual maturity commonly occurs in spring or summer. The sexes are separate except in *Chrysaora*. The gonads are purple in young medusae and turn brown in older ones. The gonads develop on the four inter-radii, each being much folded, forming a horse-shoe shaped structure which is pointed inward.

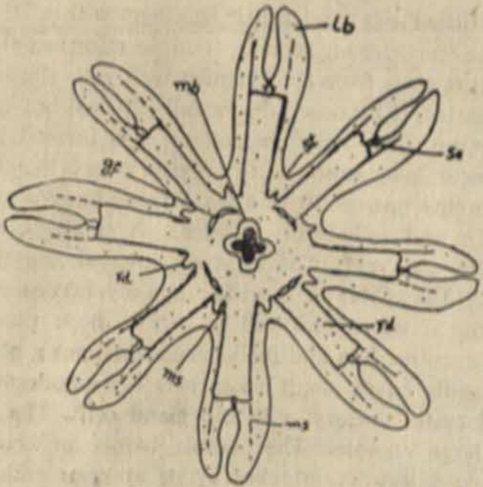


Fig. 2.9. Ephyra larva of *Aurelia* shortly after liberation.
gf—gastric filament.
lb—marginal lappet.
mb—manubrium.
ms—radial muscle.
rd—radial sac.
se—sensory organ.
st—stomach.

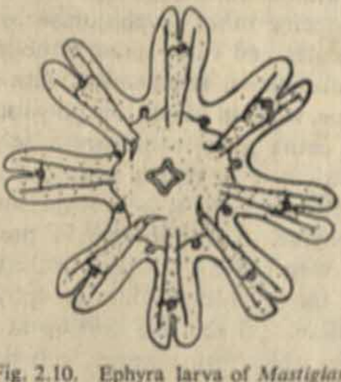


Fig. 2.10. Ephyra larva of *Mastigias*.

The spermatozoa pass through the subgenital pits into the sea water. Some of these find their way into the gastral cavities of the females where the eggs are fertilized.

The fertilized egg undergoes cleavage within the gastral cavity. The cleavages are regular, total, forming a coeloblastula. A few cells migrate from the blastular wall into the interior so that the blastocoel becomes almost full of them but later these cells disappear. The definitive endoderm is formed by a more or less typical invagination of the vegetal pole cells. As a result of this a hollow gastrula with a two-layered body wall made up of ectoderm and endoderm is formed. A small pore remains open where the vegetal wall is invaginated and this is the blastopore. The spherical gastrula gradually becomes oval, the anterior end is wider and the posterior more pointed. The ectoderm acquires cilia and the gastrula becomes a planula.

The planula has a small blastocoel. The ectoderm gives rise to nematocysts, sensory cells and gland cells. The endoderm contains large vacuoles. The planula swims for about four to five days, loses its cilia, attaches by its anterior end. The cells at this end secrete a pedal disc which helps in getting a firm hold to the substratum. This develops into a typical Scyphistoma larva of a trumpet form with basal stalk, pedal disc and expanded oral end with mouth, manubrium and tentacles. The mouth breaks through at the side of the closed blastopore. The first tentacles are perradial followed by interradial and then adradial ones. The Scyphistoma, in species inhabiting temperate zones remains without further change through fall and winter, feeding and producing other Scyphistomae by various asexual processes. In winter and early spring strobilation sets in. In some genera, strobilation is monodisc, with only one ephyra forming at a time, while in others it is polydisc strobilation. In these cases the entire polyp undergoes a series of successive transverse constrictions so that it resembles a pile of saucers diminishing in size aborally. A large scyphistoma of *Chrysaora* forms 13-15 ephyrae. A scyphistome in process of polydisc strobilation is often called a strobila. In the transformation of the oral end of the scyphistoma into an ephyra the following changes take place: (a) rhopalia develop at the bases of the primary tentacles which then together with the other tentacles are cast off or absorbed; (b) the margins of the septa thicken and give rise to the gastral filaments; (c) the margin at the site of each rhopalium grows out into a bifurcated lobe with the rhopalium in the fork so that each rhopalium is flanked on either side by a lappet; (d) the gastrovascular system sends into each octant a branch that bifurcates around the rhopalium; and (e) the ephyra when finished is constricted off by a muscular contraction and swims about as a tiny medusa. In monodisc strobilation, the scyphistoma generally regenerates its oral end before giving off another ephyra. In polydisc strobilation, the ephyra are released at intervals.

The scyphistomae live for several years. After a period of strobilation in winter and spring, they stop forming ephyrae and resume life as polyps, feeding and reproducing other scyphistomae asexually until the following winter when once again strobilation starts.

The newly released ephyra is a minute gelatinous creature one to a few millimetres in diameter. It has eight lobes bifurcated at their end into two lappets having a rhopalium in between them. From the centre of the subumbrella a short quadrangular manubrium hangs out. If food material is available, the ephyra expands and transforms into an adult medusa. In the process new tentacles are formed, on each adradius and their number continues to increase. More marginal lappets are also formed on the adradial regions. The manubrium develops long oral arms, the subgenital pits, gastrovascular system, gonads and other structures and increases in size. The young medusa which has completed this transformation is only about 1 cm in diameter. From this point on, it increases in size. The radial canals become more complicated, the gastral filaments increase in number and the oral arms develop elaborate frills and folds.

The development of the genus *Pelagia* differs from the other Semaeostomes. The planula develops directly into an ephyra without alternation of generations. The planula is ciliated. The mouth is on the lower side. The endoderm is formed by invagination. The blastocoel transforms into a large gastral cavity. Eight lappets are formed first on the lower side, these soon become eight pairs and rhopalia develop in between them. By this process the larva changes directly into an ephyra without passing through a scyphistoma stage.

The development of the species *Mastigias papna* belonging to the order Rhizostomae, which is found in Japan is well investigated. The early development of this species closely resembles that of *Aurelia*. It has monodisc type of strobilation. There is no stack of discs like that found in *Aurelia*. The transformation of the ephyra into adult is highly characteristic. The edges of the four lips around the mouth opening spread horizontally, developing a fringe of small tentacles (Fig. 2.11). Each adradial portion becomes deeply indentated, while shallow constrictions appear in the perradii (Fig. 2.11). These arms later bifurcate again, acquiring an extremely complex structure as the bifurcation is repeated many times. During this elaboration of the manubrium, the central mouth is closed off, and many small suctorial mouths appear among the fringes of the oral arms, connected by branchial canals which are subdivisions of the gastrovascular cavity formed by the repeated bifurcations of the oral arms (Fig. 2.11).

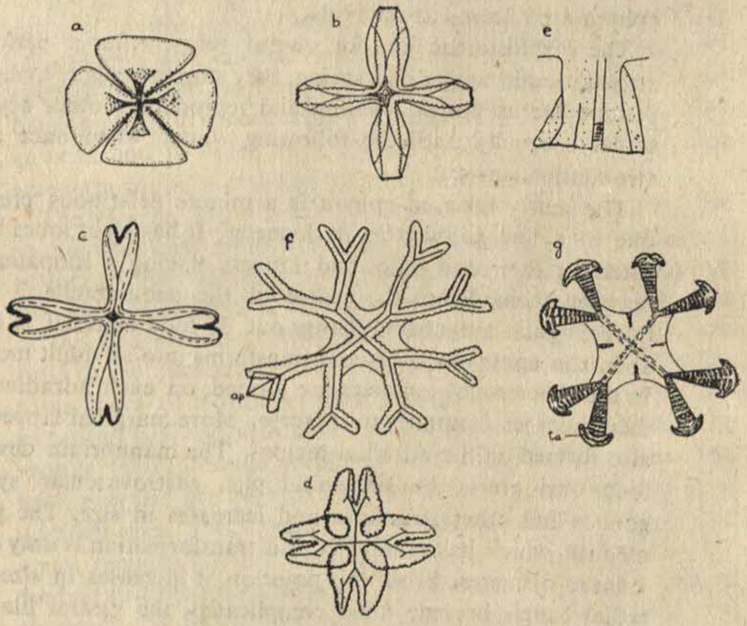


Fig. 2.11. Process of manubrium formation in *Mastigias*.
ap—opening of oral arm.
ta—rhopalium.

ANTHOZOA

GENERAL DEVELOPMENT

The Anthozoa do not have a medusoid phase in their life history, consisting only of polypoid generation. Hence the development is relatively simple than those of the other coelenterata.

The gonads are endodermal in origin. In the eggs where the quantity of yolk is more, the cleavage is superficial. In the eggs with less amount of yolk the cleavage is total and radial. In most cases the endoderm is formed by invagination. The yolk remaining in the blastocoel is absorbed by the time planula larva is formed. The planula stage is free-swimming and lasts for several days. In the solitary forms the septa become highly developed. In free-moving forms, whether solitary or colonial, they have muscular bodies whereas in the sessile species a calcareous or keratinoid skeleton is formed. These skeletons are secreted by mesogleal cells which arise as modified ectodermal cells.

DEVELOPMENT OF *SAGARTIA TRAGLODYTES*

This is a European species. It breeds every year in the beginning of August, when the temperature of the sea water reaches 15°C. The sperms are shed into the sea water through the pores that are at the tips of the tentacles on the pedal disc. The mature eggs measure about 0.08-0.09 mm in diameter. They are fertilized as soon as spawning takes place and form a fertilization membrane. The first cleavage furrow appears an hour after fertilization. This is equal and total starting at the animal pole and reaching the vegetal pole. The second cleavage is perpendicular to the first forming four blastomeres. The third cleavage is equatorial forming four upper and four lower blastomeres. At this eight-celled stage a blastocoel has already formed and it increases in size as the cleavage proceeds. The cleavage continues regularly with radial symmetry and a 128-cell stage is reached by the end of about four hours after fertilization. The blastocoel is filled with a large number of yolk cells. The endoderm is formed by invagination. After this is completed the ectoderm invaginates forming the future stomodaeum. Fifteen hours after fertilization and when the endoderm is still being invaginated, the embryo becomes ciliated and the planula larva starts swimming slowly. The body becomes thinner and longer, long cilia develop at the opposite end of the mouth (Fig. 2.12). The planula swims with this end forward and later

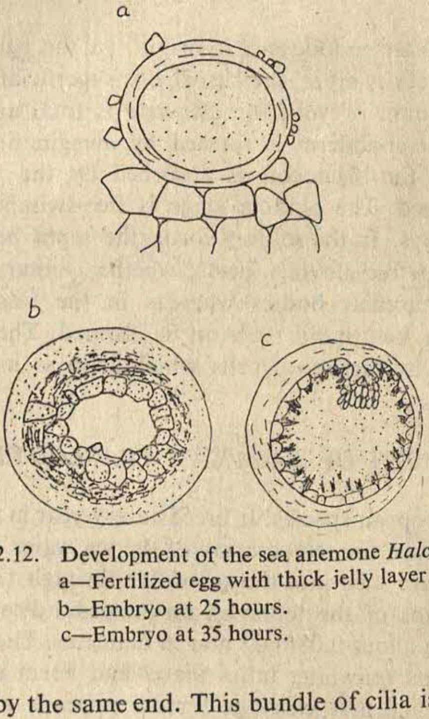


Fig. 2.12. Development of the sea anemone *Halcampa*.
a—Fertilized egg with thick jelly layer.
b—Embryo at 25 hours.
c—Embryo at 35 hours.

on attaches by the same end. This bundle of cilia is characteristic of sea-anemones. This stage is called the *Edwardsia* stage. The septa also become conspicuous at this stage. After six or seven days of free existence, the planula larva sinks to the bottom and becomes attached. The body increases in size, the tentacles appear in regular order, the number of septa rise and the gonads approach sexual maturity.

DEVELOPMENT OF *HALCAMPA DUODECIMCIRRATA*

It is a sea-anemone with an elongated body which is buried in the sand. It breeds in October and November on the coast of

Sweden. The eggs contain large amount of yolk and undergo superficial cleavage. First the males come out of the sand and shed sperms. In response to this stimulus, the females spawn. The eggs are shed from the mouth, into the sea water. After fertilization, a fertilization membrane is formed to which sand grains adhere. Endoderm formation takes place about 35 hours after fertilization. Endoderm formation is by invagination from one pole. The embryo is still solid even after the endoderm has been completely formed (Fig. 2.12) but a stomodaeum is formed and the yolk cells are absorbed. The shape of the larva changes to oval, and becomes elongated. At one end a mouth appears and the other end becomes pointed. Up to this point the development takes place inside the jelly-like case, and now the embryo starts moving inside the case. The larva finally emerges out of the sheath with the pointed end foremost (Fig. 2.13).

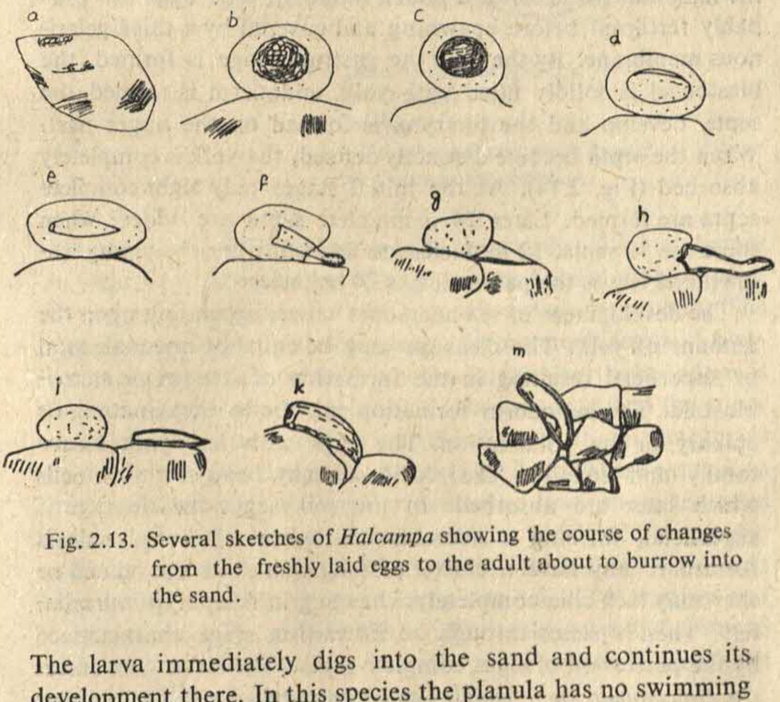


Fig. 2.13. Several sketches of *Halcampa* showing the course of changes from the freshly laid eggs to the adult about to burrow into the sand.

The larva immediately digs into the sand and continues its development there. In this species the planula has no swimming stage.

In some anemones the young develop attached to the outside of the adult, or in brood pockets or within the coelenteron. The species *Epiactis japonica* is hermaphrodite and have the young ones attached to the outer surface of the body. Eggs are formed on the younger septa and the sperms on the older septa. The eggs and sperms are shed through the pharynx together with mucous secretion as a result of rhythmical muscular contractions of the body wall. The spermatozoa measure about 47.5 μ with the head measuring only 2 μ in length. They can live for about five days in sea water at a temperature of 7.5°C. The eggs are reddish brown, large with lot of yolk; when they are extended out of the pharynx they tend to adhere to the body of the parent with the help of the mucus. Some of them which fail to stick, fall to the bottom. In the case of the eggs which have attached, the body tissue of the parent which have mucous glands, make a small invagination into which the egg sinks and the maternal tissue forms a pouch round it. The eggs are probably fertilized before spawning and covered by a thick gelatinous membrane. By the time the gastrula stage is formed, the blastocoel is solidly filled with yolk, endoderm is formed, the septa develop and the pharynx is formed on the upper part. When the septa become distinctly defined, the yolk is completely absorbed (Fig. 2.14). At the initial stages only eight complete septa are formed. Later 24 incomplete septa are added, when there are 12 septa, 12 tentacles are formed, when the young sea-anemone leaves the parent it has 24 tentacles.

The development of sea anemones varies, depending upon the amount of yolk. The cleavage may be equal or unequal, total or superficial resulting in the formation of coelom or stereoblastula. The endoderm formation may be by invagination, or epiboly or by polarization. The eggs with less yolk cleave totally and equally. The blastocoel may have few yolk cells which later are absorbed. In the yolky eggs the cleavage is superficial, forming a stereoblastula when a free planula is formed it may have a cluster of long cilia at its aboral end or they may lack cilia completely, changing into a polyp immediately. Then it passes through an Edwardsia stage characterized by the possession of eight complete septa. The larva then enters the *Halcampula* stage which has six pairs of septa. The number of tentacles and septa vary from one species to another.

The species *Cerianthus lloydii* belongs to the order Ceriantha-

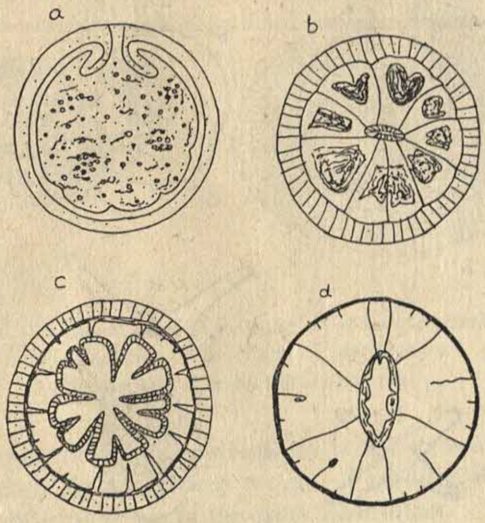


Fig. 2.14. Development of *Epiactis.*
a—Gastrula stage.
b—Septa developed.
c—Oral canal and septa formed.
d—More advanced stage.

ria. It is a north European species, hermaphrodite and breeds in February. The mature ova are spawned about an hour after the sperms are shed. Fertilization takes place externally. The eggs undergo superficial cleavage and form stereoblastula. A blastocoel appears and gradually becomes larger after the endoderm is formed. A pharynx develops. Next, the ectoderm and the endoderm together extend out in four directions with the pharynx as centre forming a rectangular figure (Fig. 2.15). These extensions are the rudiments of marginal tentacles. Two more tentacles known as oral tentacles appear at the sides of the mouth, making a total of six tentacles. Septa are formed internally and a so-called *Arachnactis* larva begins its life. The number and length of the tentacles increase, the larva elongates and sinks to the bottom and burrows into the sand where it constructs around itself a sheath of sand and slime.

Another species of Ceriantharia, *Pachycerianthus multiplicatus* has eggs containing lot of yolk. They undergo total, somewhat

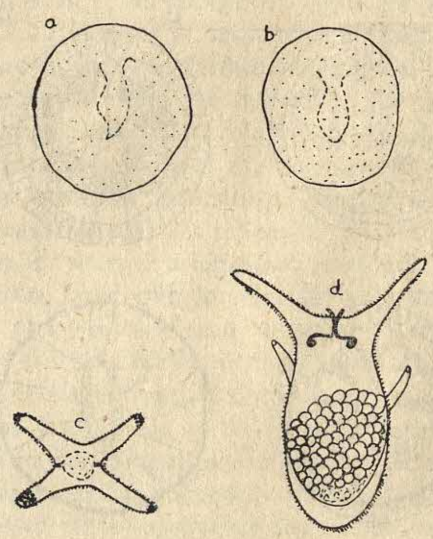


Fig. 2.15. Development of *Cerianthus*.
a, b—Successive stages in gastrulation.
c—Arachnactis stage (from above).
d—Same stage, lateral view.

unequal cleavage. It is also hermaphrodite. The sperms are shed first and then the eggs. Since the cleavage furrows start simultaneously from both the ends it is difficult to distinguish between animal and vegetal pole. Upto the eight-blastomere stage the cleavage is somewhat spiral in nature, later radial symmetry is restored by about the 16-celled stage. The centre of the blastula is solidly filled with yolk. One pole of the embryo becomes flat and invaginates forming endoderm (Fig. 2.16). The yolk in the blastocoel, becomes gradually absorbed, and the outer ectodermal cells acquire cilia. Internally the pharynx and the septa are formed and the larva starts to swim. Then the body becomes elongated, the oral end flattens out and the aboral end narrows (Fig. 2.17). The larva swims with the aboral end forward and in this position burrows into the sand with a spiral movement. The body goes on increasing in length, tentacles are formed and their number increases gradually and also the number of septa increase (Fig. 2.17). In the development of this species there is no *Arachnactis* stage.

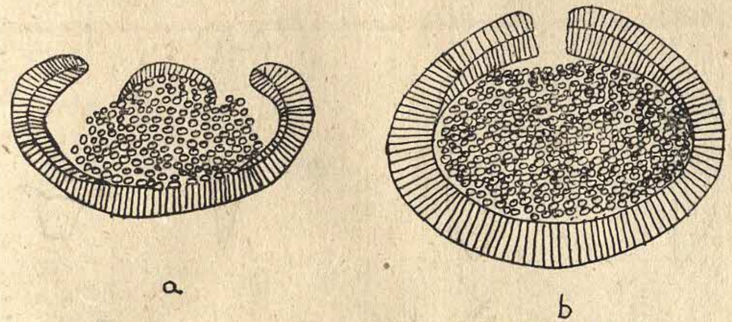


Fig. 2.16. Successive stage in gastrulation of *Pachycerianthus*.
a—Formation of endoderm by gastrulation.
b—Segmentation cavity filled with yolk.

The development of the *Antipatharia* is not known because they are mainly found in deep waters. Very little is known about the embryonic stages of the order Zoantharia.

The members of the group Madreporaria live in the warm ocean currents and many of them breed throughout the year. In many cases, the eggs are heavily laden with yolk, the cleavage is superficial. Gastrulation is by invagination. The planula larva is either pear-shaped or oval, they are often brown because of the symbiotic algae (zooxanthellae) living inside them.

The planula fix by their aboral end and change to the polypoid form, developing septa and pass through Edwardsia and Halcampula stages. The characteristic feature of these species is that there is less of muscle tissue and formation of exoskeleton. Extensive colonies are built up by asexual reproduction. Formation of exoskeleton goes hand in hand with the development of septa. The young polyps have a strong tendency to aggregate which leads to the formation of colonies.

The members of the genus *Fungia* exhibit an alternation of generations. In this genus the fertilized egg cleaves and forms a planula, which attaches and develops into a branched trophozoid. This by budding gives rise to *anthoblasts*. The periphery of the anthoblasts expand and undergo repeated lateral fissions forming the larva called as *anthocyanthus*. This larva develops into the adult *Fungia*, which reproduces sexually as well as by budding.

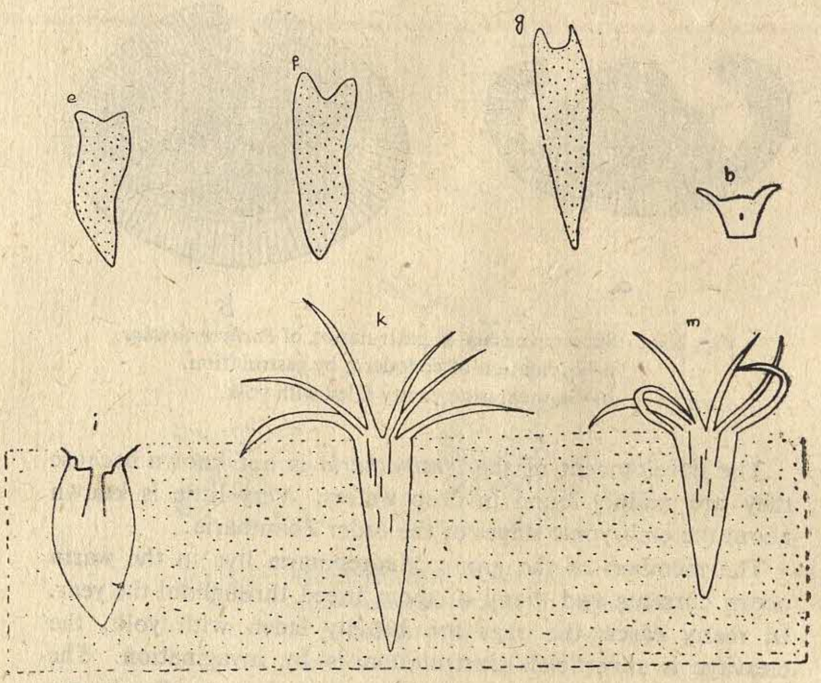


Fig. 2.17. Later development of *Pachycerianthus*.

In the group Hexacorallia the development is not thoroughly investigated. The available data indicates, that the eggs are yolky, and the cleavage superficial. A stereoblastula is formed and the yolk present in the blastocoel is gradually absorbed as the development proceeds. The endoderm is formed by delamination. The planula is ovoid or pear-shaped and free swimming. Eventually it loses its cilia and attaches by its aboral end. After this, pharynx forms by invagination, tentacles appear around the oral disc and septa develop internally. Then asexual reproduction takes place and a colony is formed.

The characteristic features of the development of the Anthozoa are: (1) the eggs are large and yolky, (2) the cleavage in many cases superficial, (3) a free swimming larval stage is absent and the planula develops directly into a polyp, (4) the polyp forms a pharynx and septa, and (5) in some groups musculature is predominant while in others calcareous or horny skeleton is characteristic.

3. *Ctenophora*

All the ctenophores are hermaphrodite and may have two periods of sexual maturity, one in the larva and a final one in the adult. The gonads degenerate between the two phases. Ovaries and testes arise along the whole length of the meridional canals. The ovaries face the principal (tentacular and pharyngeal) plane and the testes on the interradial sides.

The ctenophores as well as their eggs are planktonic. The eggs of *Bolina* are smaller when compared with the eggs of *Beroe* which measure about 1 mm in diameter. In both the cases the eggs are transparent. The eggs of *Beroe* have been studied because of their advantageous size. The egg is surrounded by jelly and protected by a very thin membrane which thickens after fertilization. According to Yastu three visible concentric formations are distinguished in the egg, an extremely thin homogeneous outer layer, the ectoplasm and the endoplasm (Fig. 3.1).

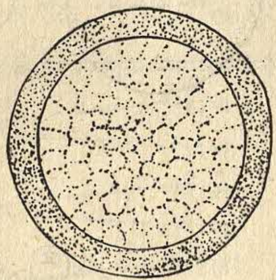


Fig. 3.1. *Beroe ovata* egg with its three concentric constituents: the thin homogeneous outer layer, the fluorescent ectoplasm (fine dots) and the alveolar endoplasm.

The outer layer is semi-fluid without granules and is difficult to observe. The ectoplasm is rather thick having a fine alveolar structure. The endoplasm has a coarse alveolar structure.

Since the great majority of the larvae in these species are planktonic, development takes place as they float in the sea.

In the genus, *Coeloplana* the eggs are ejected from the mouth and develop in the space between the underside of the flattened body and substratum. In *Lyrocties* and *Tjalfiella*, lateral chambers are formed by the distension of the meridional canals. The eggs are fertilized and develop to larvae within these brood pouches as in viviparous animals. These modifications have taken place in response to the various special habits of the species.

Cleavage is regular and two equal blastomeres are formed by the first cleavage. The first cleavage plane coincides with the future pharyngeal plane, and the second plane corresponds to the tentacular plane (Fig. 3.2).

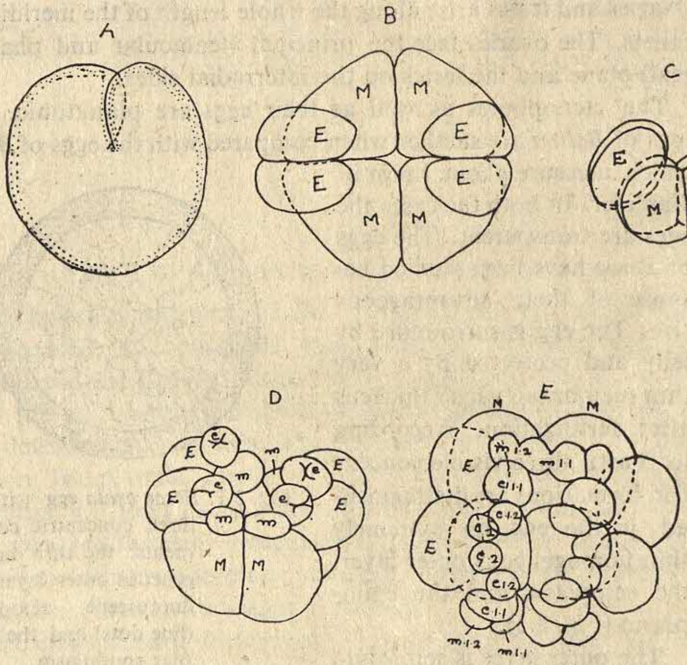
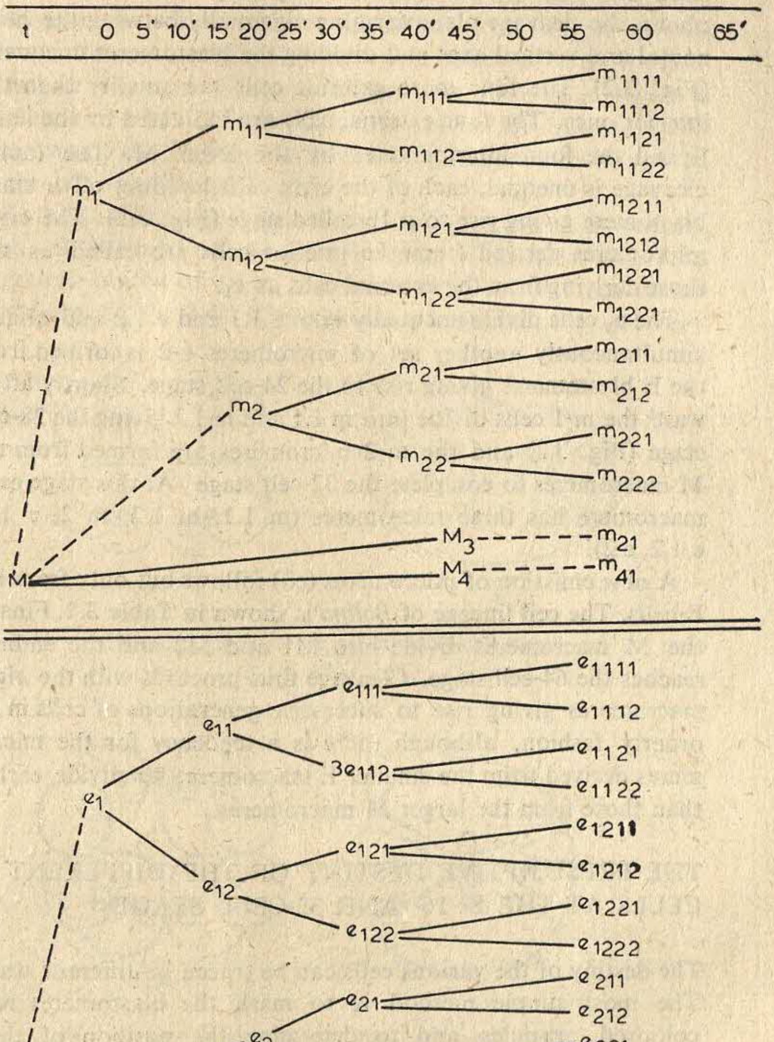


Fig. 3.2. Early development of *Coeloplana*.
A—Mid-cleavage.
B—8-cell stage from animal pole.
C—8-cell stage from side.
D—Transition from 16-20 cell stage.
E—28-cell stage from animal pole.

TABLE 3.1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e221
e22
e2222
E
e3
e31
e32
Ml micr.
2
12
20
24
36
52
80
112
Mi macr.
2
12
16

These two cleavage planes divide the egg into four equal sized blastomeres. The third cleavage is peculiar to the ctenophora, the cleavage plane forming diagonally between the horizontal and vertical axes and dividing the blastomeres unequally (Fig. 3.2). The four more exterior cells are smaller than the interior ones. The four external cells are indicated by the letter E and the four interior ones by the letter M. The fourth cleavage is unequal, each of the eight cells budding off a small blastomere giving rise to a 16-celled stage (Fig. 3.2). The eight micromeres derived from the interior cells are called as m_1, those deriving from the external cells as e_1.

The e_1 cells divide unequally into e 1.1 and e 1.2 and almost simultaneously another set of micromeres e-2 is formed from the E blastomeres giving rise to the 24-cell stage. Shortly afterward the m-1 cells divide into m 1.1 and m 1.2 giving the 28-cell stage (Fig. 3.2) and the m-2 micromeres are formed from the M macromeres to complete the 32-cell stage. At this stage each macromere has three micromeres (m 1.1, m 1.2, m 2, e 1.1, e 1.2, e 2).

A new emission of micromeres (e3) follows but only from the E-cells. The cell lineage of *Bolina* is shown in Table 3.1. Finally the M macromeres divide into M1 and M2 and the embryo reaches the 64-cell stage. Cleavage thus proceeds with the eight macromeres giving rise to successive generations of cells in an orderly fashion, although there is a tendency for the micromeres derived from the smaller E macromeres to divide earlier than those from the larger M macromeres.

THE PRESUMPTIVE DESTINY OF THE DIFFERENT CELLS AT THE 8- 16- AND 32-CELL STAGE

The destiny of the various cells can be traced in different ways. The most simple method is to mark the blastomeres with coloured granules and to determine the position of these granules in the developed larva.

Suppose a granule is placed at the animal pole in the immediate vicinity of the polocytes. At the end of development this granule can be seen at the bottom of the gastral cavity in the larva. This indicates, that the blastopore and later the larval mouth forms at the animal pole. A granule on the opposite pole does not move much. It remains on the surface and is finally found on the apical organ which forms at the vegetal pole of the egg. A granule which has been put on an E cell is found at the larval stage in a gastro-vascular canal, while a granule on an M-cell is found in the gastric sac. According to these results the E gives rise to the longitudinal canals, the M to the gastric pockets.

At the 16-cell stage, a granule placed upon an e_1 micromere (Fig. 3.3) is found on the ciliated combs of the larva (Fig. 3.3). A granule placed on an m_1 micromere (Fig. 3.4) is found on the apical organ (Fig. 3.4). These results suggest that m_1, contrary to the opinion held in the past, have nothing to do with the formation of the ciliated plates. One granule placed on an e_{11} and another on an e_{12} are found in the larva on ciliary plates but on different rows. This suggests that every e_1 (from which e_{11} and e_{12} derive) is involved in the development of the rows of ciliated plates.

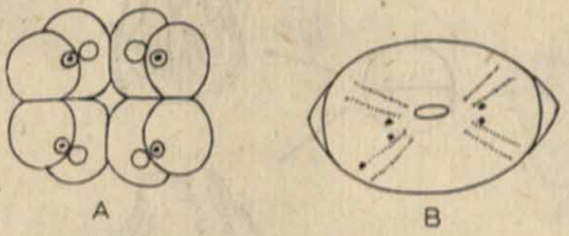


Fig. 3.3. *Beroe ovata* egg at the 16-cell stage.

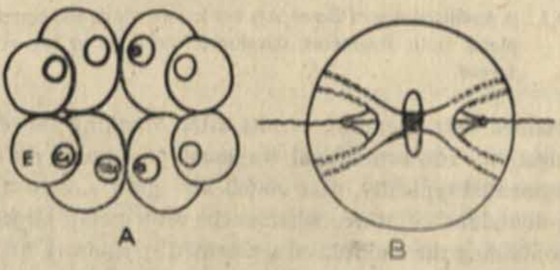


Fig. 3.4. *Beroe ovata* granules on the apical organ of the larva.

The micromeres given from macromeres at gastrulation at the animal pole have a completely different destiny. The derivatives from M give rise to mesenchyme and those of the E to the larval musculature.

The fragmentation of the unfertilized egg (*Beroe*) has been performed by Yastu (1911). He sectioned the egg into two parts and then fertilized the fragments. Each fragment gave rise to a small but otherwise normal larva (Fig. 3.5). La Spina (1963) also obtained corresponding results after breaking the egg by centrifugation. The centrifugal fragment containing the ectoplasm segmented typically, gastrulated and gave rise to a larva having abundant ciliature, whereas the centripetal, larger fragment containing the endoplasm segmented irregularly and gave rise to an undifferentiated cellular mass lacking in cilia (Fig. 3.6). These results emphasize the importance of the ectoplasm for the development of the ciliary plates.

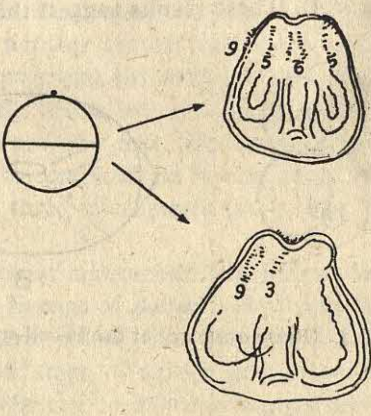


Fig. 3.5. A fertilized egg of *Beroe* was cut in two along the equatorial plane: both fragments developed into more or less normal larvae.

Experimental isolation of the blastomere was done by Chun (1880). He collected fresh eggs from the sea after a storm and sometimes noticed two half larvae in the same capsule each having only four rows of ciliary plates instead of the usual eight. Chun supposed that these half larvae had derived from the two

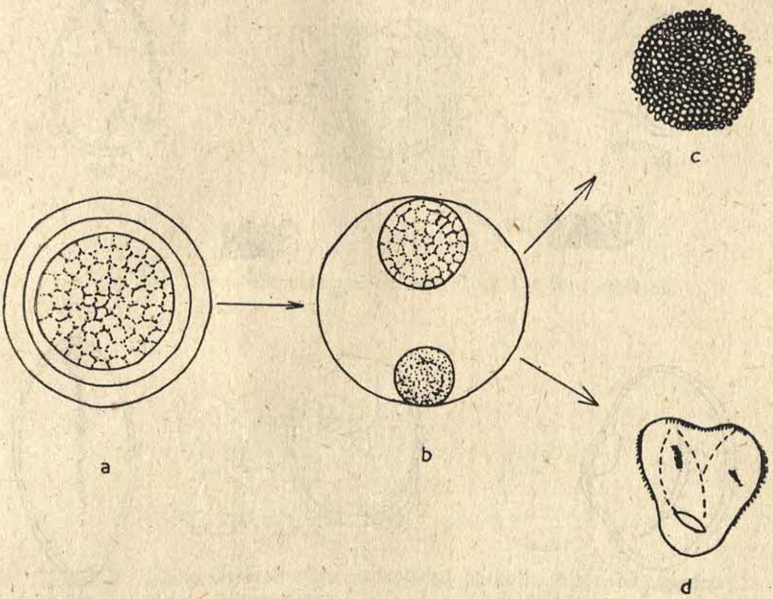


Fig. 3.6. An egg of *Beroe* (a) was broken by centrifugation (b) the small centrifugal fragment contains the green plasm develops into a more or less ciliated larva (d) the antripetal fragment segments irregularly and gives rise to a cellular mass (c).

mechanically isolated blastomeres, a hypothesis he confirmed by experiments.

The isolation of blastomere from eggs at the eight cell stage was also done by Yastu (1912) (Fig. 3.7). From a simple E-cell he obtained a larva with only one row of comb-plates. From two isolated cells he got a larva with two rows of comb-plates. From two M-cells he got larvae of various types. In one of them two comb-rows were formed, in another embryo a very small group of comb-plates was developed. In three no comb-plates were formed at all. According to Yastu the developmental potencies of the E and M cells at least with regard to the formation of the comb-plates are the same. Each cell is responsible for one row of comb-plates.

The combination, 1E+2E, according to the hypothesis should

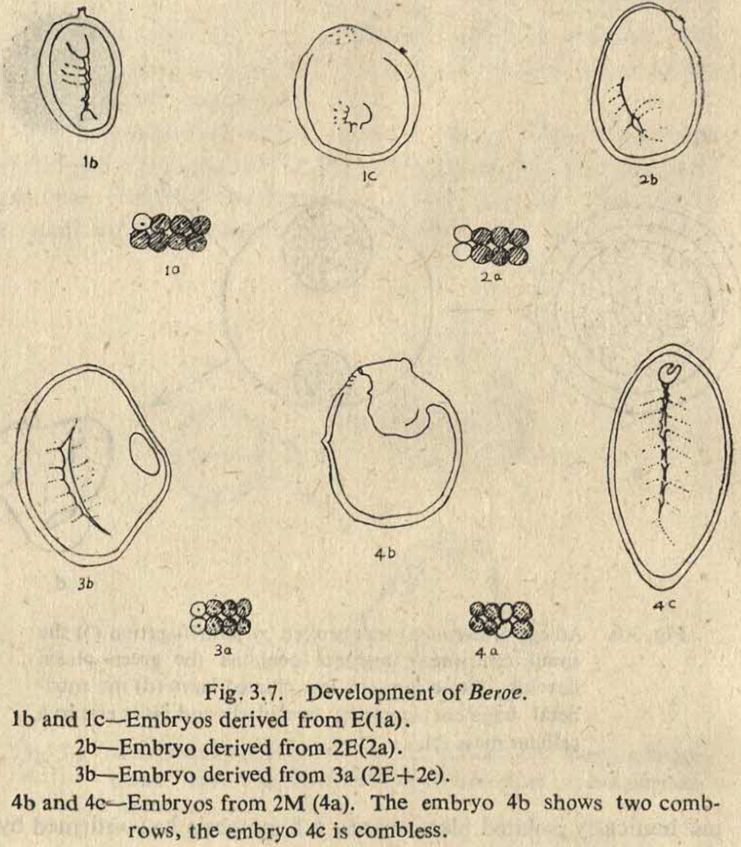


Fig. 3.7. Development of *Beroe*.
1b and 1c—Embryos derived from E(1a).
2b—Embryo derived from 2E(2a).
3b—Embryo derived from 3a (2E+2e).
4b and 4c—Embryos from 2M (4a). The embryo 4b shows two comb-rows, the embryo 4c is combless.

yield larvae with three comb-rows. However this result was not obtained.

Faraglio (1963a) did some more isolation experiments on the eight-celled stage. At this stage, he isolated all E-cells of the same egg and followed their development (Fig. 3.8). The development was good, the cells continued to segment as though *in situ* and finally gave rise to four partial larvae each having two comb-rows.

The isolated M-cells do not develop easily. This is probably due to the fact that the M-cells, produce fewer micromeres than the E and so remain uncovered. In any case, the larvae which developed did not have any combs (Fig. 3.9).

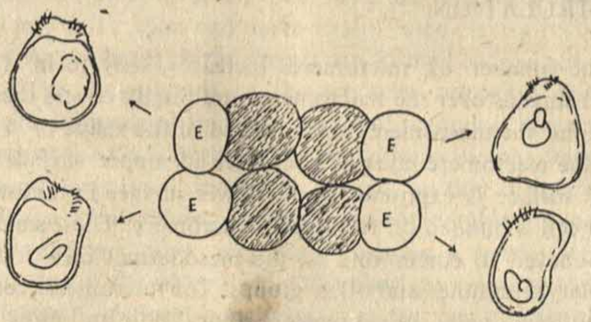


Fig. 3.8. The four embryos derived from the four isolated. E-cells.

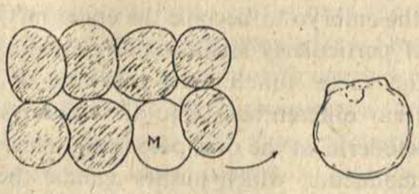


Fig. 3.9. Larva derived from an isolated M-cell. Note the absence of the ciliated plates.

Isolation of 1E+4M: the larvae has only two comb-rows.
Isolation of 1E+2M: the larvae obtained from this combination has only two comb-rows.
Isolation of 2E+1M: resulted in larvae with four comb-rows.

These results suggest that only the E-cells and not the M-cells, are involved in the formation of the comb-rows, each of them giving rise to two comb-rows. This conclusion is confirmed by the results obtained after killing cells of the egg at the 16-cell stage (Farfaglio, 1953b). Since the m_1 are not involved in the genesis of the comb-rows, what is their function? Ortolavi (1964) removed all the m_1 cells and obtained larvae with an apical organ, defective in respect of some structures, the long cillia forming the protective dome were still present, but the four pillars supporting the otholite and the short cillia of the pit were absent. Probably the m_1 are responsible for formation of the ciliary pillars, each m would be responsible for one pillar.

GASTRULATION

As the number of micromeres increases, they form a layer which spreads over the macromeres and finally covers them. By this time the macromeres are arranged in the shape of a bowl, with the macromere layer as a lid over its upper surface while lower surface is exposed. On the lower surface in this stage, a small cell is budded off from each macromere. These small cells are believed to correspond to the mesodermal cells found in the platyhelminthes and other groups. The micromeres continue to multiply, finally spread over the aboral pole and grow down as a one-layered sheet over the macromeres. The macromeres also invaginate into the interior so that the gastrula arises by the combined processes of epiboly and emboly. As the micromeres cover the embryo to become the epidermis, four interradial bands of particularly small, rapidly dividing cells become noticeable and these differentiate into the comb-rows. The aboral ectoderm differentiates into the statocyst and related parts. The ectoderm at the oval pole invaginates extensively to form the stomodaeum, which pushes against the entoderm in such a way as to constrict it into four pockets, two on each side of the tentacular plane. From them the gastrovascular canals arise by active entodermal outgrowth. The tentacle sheaths that possess them originate as ectodermal invaginations from whose base the tentacle sprouts. The embryo now escapes as a free-swimming *cydippid* larva. This cydippid larval stage occurs in the development of all the ctenophores, being common to all the orders except that in the Beroidea. The tentacular and pharyngeal axes are of approximately the same length and the pharynx is circular in cross section.

The larva swims in the sea by moving its comb-plates. The changes that take place during metamorphosis are, the number of comb plates increase, the cilia grow longer, the tentacles lengthen, branch and become freely movable in and out of their sheaths. The water vascular system develops completely and the general shape of the body gradually changes toward that of the adult.

In the Lobatea the whole body of the cydippid larva shortens in the tentacular axis and lengthens in the pharyngeal direction. The lower parts of the body in the pharyngeal plane are prolonged into conspicuous sleeve like structure and the water canals grow into this region and make complicated ziz-zag revolutions there. At the bases of the sleeves are formed ear-shaped projections. The main cones of the tentacles retrogress and their branches elongate to form the so-called secondary tentacles.

Among the Cestida the difference between the pharyngeal and tentacular axis is still more pronounced, so that the body takes the shape of a ribbon. The four rows of comb-plates adjacent to the pharynx grow very long, forming a border along the upper edge of the ribbon while the plate rows next to the tentacles become vestigeal consisting of a few comb plates situated near the centre of the upper margin. The secondary tentacles hang from the lower margin of the ribbon.

In the *Coeloplana*, the mouth of the cydippid larva widens, and the metamorphosing larva moves over the substratum by means of the cilia on the stomodaeal surface. Shortly afterward, the comb plates are shed, pigment cells develop and the whole body becomes flattened so that it resembles a turbellarian. The body elongates in the tentacular axis and the tentacle sheath regions extend upward like chimneys, so that the general shape of the body is somewhat like a harp. In most of the species, of the order, the water canals form a highly ramified network.

The form of the adult in the order Beroidea is not very different from that of the cydippid larva, except for the complete absence of tentacles in all stages and the markedly reticulate water vascular system.

4. *Platyhelminthes*

INTRODUCTION

The chapter deals only with the development of the single class Turbellaria among the phylum of Platyhelminthes. The embryology of planarians differs markedly from that of every other group; cleavage produces an irregular shaped group of loosely associated cells that may not touch each other and development proceeds without the formation of germ layers and without gastrulation. Most of the yolk, is external to the embryo in yolk cells.

Since the power of regeneration of adult planarians is so great and so much has been worked on, the experimental embryology of planarians is of considerable interest.

REPRODUCTION

Sexual reproduction begins when the reproductive organs are fully developed. All the platyhelminthes without exception are hermaphrodite. Self-fertilization does not take place. There is a well-developed copulatory pouch or seminal receptacle and a true copulation takes place between any two mature individuals. In the groups, polyclads, some freshwater triclads and rhabdocoels the spermatozoa are bundled into spermatophores which are placed in the seminal receptacle of the partner. A simple method of copulation called hypodermic injection occurs widely in many species. In this method, one animal punctures the epidermis of the other by means of the copulatory apparatus and injects its spermatozoa into the parenchyma. The sperms travel through the tissues and finally enter the uterus, where fertilization takes place.

One conspicuous characteristic of the platyhelminthes is their possession of a special gland, called the yolk gland which forms yolk cells. These are combined with the egg cells to make up composite eggs. In the groups Acoela, Polycladida, and some species of the Rhabdocoela and Allocoela do not form composite eggs. These eggs are commonly called as simple eggs. The composite eggs are also called as *ectolecithal* eggs because the yolk is accumulated outside the egg cell and the simple eggs are known as *entolecithal* since the yolk is situated within the egg cell.

In the ectolecithal egg, yolk granules surround the ovum and the entire egg is covered by an egg shell which is secreted by the shell gland. Within one shell there may be included as many as several thousand yolk cells and from one to ten egg cells. Such eggs are generally large in size.

In simple eggs, several of them may be surrounded by a thin shell, which is in turn enclosed in a gelatinous substance. The eggs are deposited in a single layer making up egg plates of various shapes. There are conspicuous differences in the mode of development between simple and composite eggs. The development of the simple eggs follows a primitive state, whereas that of the composite eggs is subjected to extreme modifications because of the existence of the yolk.

DEVELOPMENT OF ENTOLECITHAL EGGS OF POLYCLADIDA

In the Polycladida the cleavage is of the spiral determinate type and so closely resembles that of annelids and molluscs. The size of the ovum varies with the species. In the species, *Planocera reticulata* the diameter of the ovum is 310–330 μ. The average diameter of the egg is between 130 and 150 μ as in *Notoplana hermitis*. The eggs may be as small as 88–95 μ as in *Stylochus uniporous*. If the egg is more yolky, then the development proceeds at a slower rate.

The egg divides equally and totally and the first cleavage plane cuts vertically through the animal and vegetal poles and divides the egg into two nearly equal sized blastomeres (A B C D). The second cleavage is also vertical, but unequal, dividing the blastomeres into two fairly large (B, D) and two smaller ones (A, C). At this time the blastomere D is the largest and its position indicates the future posterior side of the embryo. As a result of this the blastomere B marks the anterior end and if viewed from the animal pole, the A blastomere lies on the left and blastomere C towards the right. The blastomeres A and C are in contact at the animal pole. While B and D make contact at the vegetal pole forming the so-called cross furrow. The third cleavage is horizontal and unequal, dividing the blastomeres in a strongly dexiotropic direction. In general, the larger blastomeres divide at a faster rate. In this case the blastomere D divides first followed by B, C and A. The blastomeres which are cut off towards the animal pole are smaller (micromeres) and towards the vegetal side are larger (macromeres). The fourth cleavage (8- 16-cell stage) laevotropic first, the macromere at the vegetal side divide forming the second quartet of micromere (2a—2d), but before these are completely separated the first micromere quartet divides into la^{2}—ld^{2} on the vegetal side and la^{1}—ld^{1} on the animal pole side. la^{2}—ld^{2} correspond to the trochoblasts of annelids and molluscs.

The fifth cleavage (16- 32-cell stage) is dexiotropic leading to the formation of a typical spiral cleavage of the 32-cell stage. First, 2D and 2B give off 3d and 3b dexiotropically. There is a short time lapse before 2A and 2C separate off 3a and 3c. In the meantime 3d and 3b divide dexiotropically to form 2d^{1}, 2b^{1} and 2d^{2}, 2b^{2}. This time, 2d^{1}, 2b^{1} at the animal pole side are smaller than 2d^{2}, 2b^{2} on the vegetal side. While the cleavage of 2d, 2b is proceeding the four blastomere la^{1}—ld^{1} also divide dexiotropically, producing la^{11}—ld^{11}. At the same time the remaining 2a, 2c micromeres divide and finally the other two macromeres 2A and 2C, give off 3a and 3c.

Thus the 32-cell stage embyro, which has first completed its fifth cleavage, consists of 16 cells derived from the first micromere quartet (1a—1d), eight cells derived from the second micromere (2a—2d), four cells comprising the third set of micromeres (3a—3d) and the four macromeres (3A—2D). Among these the third micromere quartet (3a—3d) are the smallest and the macromeres (3A—3D) are the largest. In the subsequent divisions the isochrony of cleavage is increasingly lost, so that the 32-cell stage is succeeded by a 36-cell, and then 40-, 44-, 45-, 53-, 61-cell stages.

After the embryo has reached the 32-cell stage, the next event is the cleavage of the macromeres (3A—3D) to form the 36-cell stage. This division is highly peculiar in that the 'macromeres' which are left at the vegetal pole (4A—4D), in spite of their name, are minute, while the four 'micromeres' given off at the animal pole side contain the major part of the yolk and are many times larger. The four little macromeres (4A—4D) do not divide after this. When the embryo gastrulates, they move to the interior where they later degenerate and are absorbed. The three cells, 4a, 4b and 4c also do not divide further and later degenerate. On the other hand the 4D cell at the posterior side of the embryo proceeds to divide actively becoming the source of the future endodermal as well as mesodermal tissue.

GASTRULATION

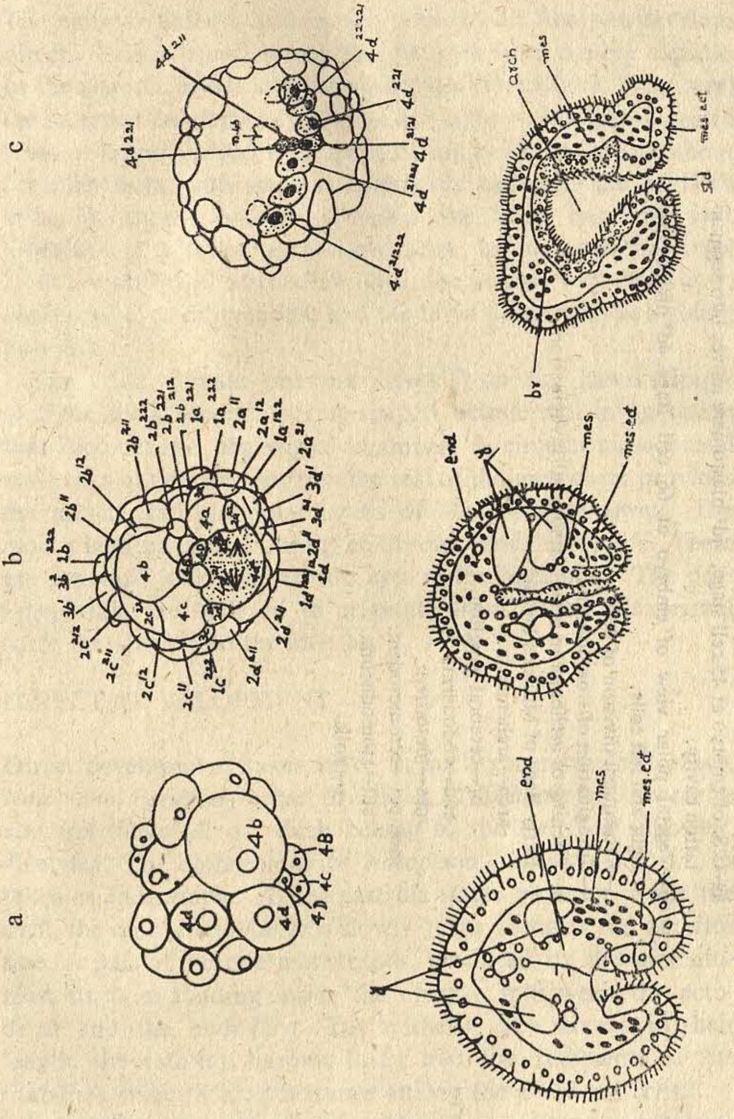
Gastrulation (Fig. 4.1) occurs chiefly by epiboly. The cells derived from the first to the third quartets of micromeres proliferate by repeated divisions and gradually extend towards the vegetal pole, enveloping the cells of this region and finally forms a stereogastrula, without a blastocoel left in its centre. The cells which fill the interior of the gastrula are chiefly the three yolk-laden blastomeres (4a, 4b, 4c) of the fourth micromere quartet and the descendants resulting from the divisions of the 4d cell. In addition there are the brain cells derived from the first micromere quartet and the ectomesoderm cells which arise from the second micromere quartet.

Before the division of the fourth micromere quartet is complete, the first micromere quartet ($1a^{11}$—$1d^{11}$) undergoes a distinctly laevotropic cleavage, forming the small $1a^{11}$—$1d^{11}$ and the fairly large $1a^{112}$—$1d^{112}$, making the 40-cell stage. These $1a^{111}$—$1d^{111}$ cells were designated as the apical cells by Selenka (1881) and they are believed to invaginate and proliferate to give rise to the brain and the organs of the forebrain (Kato 1940). According to the investigations of Surface (1907) on *Holoplana* (Table 4.1) all the cells of the first micromere quartet lead the other blastomeres into repeated cleavages and finally their descendants are arranged in a spherical formation around the apical cells at the animal pole, thus making up the main part of the anterior dorsal ectoderm of the larva.

The descendants of the second micromere quartet on the other hand, chiefly form the ectoderm of the posterior ventral part of the embryo. Of these the four cells $2a^{222}$—$2d^{222}$ in particular are clearly located at the vegetal pole, in close contact with the macromere quartet, and when the embryo gastrulates and finally forms a pharynx, these cells are believed to take part in its development. When $2a^{11}$—$2d^{11}$ divide, the rather large blastomeres $2a^{112}$—$2s^{112}$ derive from them more into the interior of the embryo and develop into ectomesoderm. The descendents of these cells become located around the mouth, and later form the musculature around the pharynx.

The cell-lineage of the 4d blastomere in the polyclads is complicated, and specialized to a certain extent (Table 4.1). About the time when the apical cells appear and the second micromere quartet is beginning to cleave (44-cell stage), the 4d blastomere forms a mitotic figure at right angles to the surface of the embryo and cleave into a large and small cell. The large cell ($4d^1$) remains at the surface of the embryo while the small one ($4d^2$) moves into the blastocoel (Fig. 4.1). According to Surface, $4d^1$ which remains at the surface is the endoderm mother cell (entoblast) while the $4d^2$ cell which migrates inward is the mesentoblast, which also gives rise to endoderm. Next each of these cells divides to form the left right pairs, $4d^{21}$ and $4d^{22}$, as well as $4d^{11}$, $4d^{12}$ (Fig. 4.1). The $4b^2$ daughter cells divide again into large and small cells, the smaller ones become endoderm, while the larger ones ($4d^{212}$, $4d^{222}$) continue to divide repeatedly and become the source of the mesoderm (Fig. 4.1).

The daughter blastomeres of 4d, which remain on the surface of the embryo, continue to divide and send, more endoderm cells into the interior, but at about this time, these blastomeres together with the other micromeres of the fourth quartet (4a, 4b, 4c) become covered by the ectoderm cells as the result of the epiboly which is taking place. The endoderm cells, which have been multiplying inside the embryo, form a clump located at the lower side near the 4b cell, but no cavity of the embryo that can be called as archenteron has not yet appeared. Near the original blastopore which closes and disappears, an ectodermal invagination forms a pharynx, that connects with the entodermal mass derived from $4d^1$; this mass hollows out to


a
4d
4d
4b
4B
4c
4D
b
4b
4c
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4d
f
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e
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mes
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become the intestine. The axial relations shift in the meantime. The apical pole bearing the frontal gland moves forward and becomes the anterior end and the mouth region shifts backward. The embryo flattens and in the majority of Acotylea develops directly into a small worm that escapes from the egg capsule. In the group Cotylea and some Acotylea (Holoplana, Planocera) the flattened ciliated embryo puts out eight posteriorly directed lobes or lappets edged with specially long cilia and swims about for a few days. This stage is commonly called as the Muller's larva. In some species of *Stylochus*, the larva has only four lobes and it is called as Gotte's larva. In any case, the larval lobes are absorbed after a few days, the sensory tuffs disappear, additional eyes differentiate and the larva flattens out as a young polyclad.

The adult plicate pharynx arises from the larval simple pharynx by a secondary ring-shaped ectodermal invagination that becomes the pharyngeal chamber. A circular mesodermal mass that has separated from the rest of the mesoderm provides the muscle and glandular layers of the adult pharynx. The mouth is often closed during embryonic and larval life. There are generally only one or two eyes in the beginning. The protonephridia are believed to originate from paired ectodermal cords that grow into the interior.

DIRECT DEVELOPMENT

Direct development takes place in such genera as *Leptoplana*, *Notoplana*, *Pseudostylochus*, *Stylochus*, *Stylochoplana*, *Discocoelus* and *Holoplana* all of which belong to the polyclad suborder, Acotylea. The embryology of *Notoplana hermilia* (Fig. 4.2) is taken as an example. As the gastrula starts rotating inside the shell, the animal-vegetal axis slowly turns towards the anterior side. A pair of mesodermal strands, produced by the mesentoblast starts expanding inside the embryo inbetween the ectoderm and the endoderm. The epithelial cilia increase in their length, the rotation become faster and the rudiments of the rhabdites make their appearance among the ectoderm cells.

Internally, the yolk laden cells of the fourth micromere quartet fuse, their cell boundaries are lost and form a single mass. This single mass divides into a large number of cells

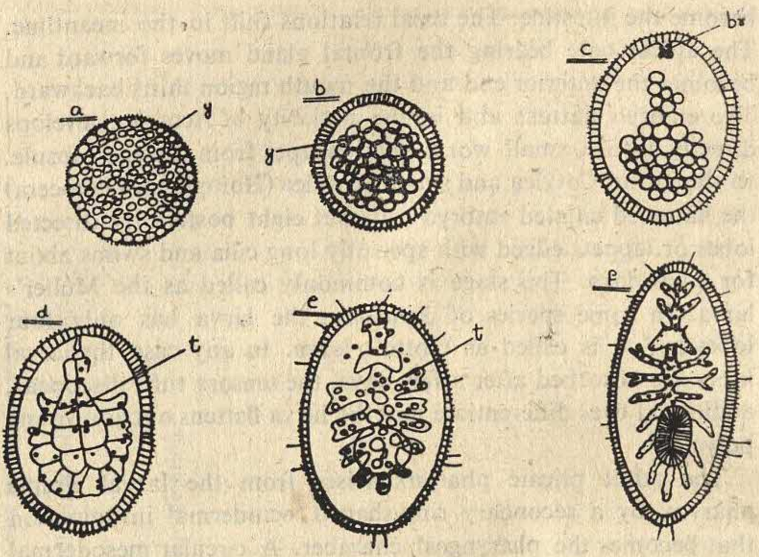


Fig. 4.2. Direct development of *Notoplana*.
a—Gastrula.
b—Yolk in interior of embryo divided into small clumps.
c—Beginning of ectodermal invagination from anterior end of embryo.
d—2-eyespot stage.
e—4-eyespot-stage, shortly before hatching.
f—Embryo immediately after hatching.
br—cerebral ganglion.
i—intestine.
y—yolk

(Fig. 4.2). The endodermal cells proliferate, surround the yolk cells, and send processes into it, through which finally all the yolk is absorbed. The embryo bends anteriorly, so that the aboral pole shifts to its front end and the oral pole finally takes up a position near its posterior end. At this stage the embryo becomes oval in shape, a pair of eye-spots appear in the ectoderm near the aboral pole. These eventually sink from the surface into the mesodermal layer. At this stage, the rudiments of the brain and frontal glands make their appearance at the aboral pole.

The endoderm with continuous development becomes sac-shaped and undergoes great ramification. The mesoderm becomes well developed at the anterior end of the embryo. It also forms a mass at the posterior end which becomes the pharynx rudiment. During this stage, the ectoderm at the region of the former blastopore, invaginates in the form of a slender cord which passes through the pharynx rudiment and reaches the lumen of the intestine. A split appears in this cord, which is connected with the intestinal space. This entire structure becomes tubular and develops into the epidermis of the pharyngeal sheath. As the development proceeds, annular folds arise in the pharynx rudiment and the pharyngeal sheath is formed. Within another week, the two eye-spots become divided into two pairs, the frontal gland is clearly defined, the musculature of the pharyngeal gland assumes a radial arrangement. A number of immobile sensory filaments appear around the edge of the body. Hatching takes place by about 21 days. The frontal gland helps the embryo break through the egg shell. The mouth opening appears five days after hatching.

INDIRECT DEVELOPMENT

The species which undergo indirect development (Fig. 4.3) forming a Muller's or Gotte's larva are restricted to the polyclad suborder Cotylea and a few species of Acotylea.

It was Johannes Muller (1850) who first discovered that the Muller's larva represents a developmental stage of the Turbellaria. In the genus, *Planocera multitentaculata* the development is similar to that of the directly developing forms. Five days after spawning epiboly is complete and the blastopore is closed over. On the sixth day, an ectodermal invagination takes place in the vicinity of the original blastopore and a short, broad pharynx connection with the intestine is formed, the inner surface of the pharynx and intestine develop cilia. The aboral pole invaginate giving rise to the rudiments of brain and frontal gland. A single eye spot, rudiments of rhabdites in the ectoderm appear. The embryo bends anteriorly and a bundle of sensory filaments, the apical sensory tuft, appears at the anterior end. Internally the yolk mass is subdivided into small cells (Fig. 4.3). Next the central side of the embryo become flattened and longitudinal depressions appear at the sides of the mouth. The single antero-ventral process, a

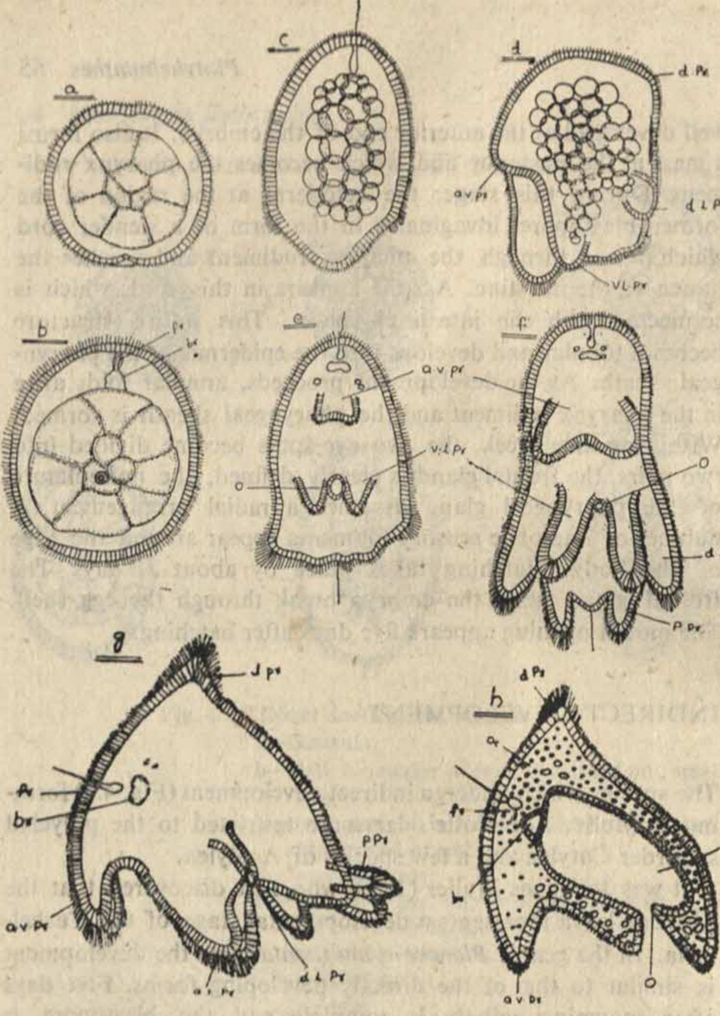


Fig. 4.3. Indirect development in *Planocera*.
a—Gastrula.
b—Formation of procerebral organ rudiment.
c—2-eyespot stage.
d—Side view of embryo at 3-eyespot stage.
e—Ventral views of same.
f—Muller's larva (ventral view).
g—The same larva side view.
h—Longitudinal section of the same.
a.v.p.r.—anteroventral process.
br—brain
d.l.pr—dorsolateral process.
d.pr—dorsal process.
fr—procerebral organs.
i—intestine.
o—mouth.
oc—eyespot.
p.pr—posterior process.
v.l.pr.—ventrolateral process.

ventrolateral process and a pair of dorso-lateral processes and a single dorsal process is formed. The cells forming these processes are tall, columnar with long cilia. It is considered most probable that these cells arise from the $2a^2$—$2d^2$ quartet.

On the tenth day, a second eye-spot is added on the right of the mid-line, and a second sensory tuft appears at the posterior end. By this time the rate of rotation increases, the left eye-spot divides as a result of which there are three eye-spots. The processes increase in size, especially the antero-ventral one, and a pair of posterior processes are added at the posterior end, the total becoming eight. This is now called as the Muller's larva.

The larva escapes from its shell by about 14th or 15th day, and begins a free swimming life. It is about 0.2-0.3 mm in length. When seen from the side it forms a triangle, with the antero-ventral process as the front angle, the dorsal process forming the top angle, and the posterior process completing the triangle. There is a circular mouth located behind the antero-ventral process. From this a narrow stomodaeum leads into a spacious intestine. Around the stomodaeum is a mass of mesoderm which is the rudiment of pharynx. There are sensory tufts on both anterior and posterior ends. The brain and the frontal glands are well developed.

According to Lang (Fig. 4.4) the larva becomes flattened as it increases in length, the various processes diminish in size and finally disappear. For the first time the larval shape begins to

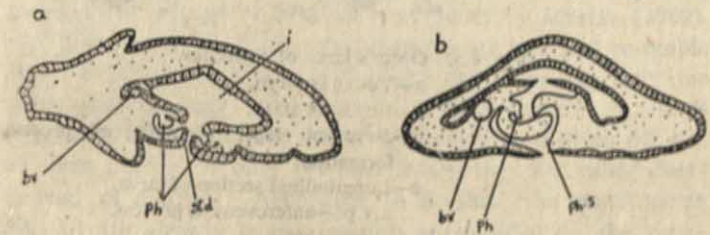


Fig. 4.4. Metamorphosis in *Yungia*.
a—Longitudinal section of Muller's larva.
b—Longitudinal section after metamorphosis.
br—brain.
i—intestine.
ph—pharynx of adult.
ph.s—pharyngeal sheath.
std—stomodaeum.

resemble that of an adult polyclad. The most striking feature of the metamorphosis is the development of pharynx. The mesodermal mass of cells which surround the stomodaeum develop into pharynx. The pharyngeal sheath arises around the protruberance. At the time of metamorphosis the stomodaeum is pulled outward and its wall forms part of the body wall.

The Gotte's larva (Fig. 4.5) resembles the Muller's larva,

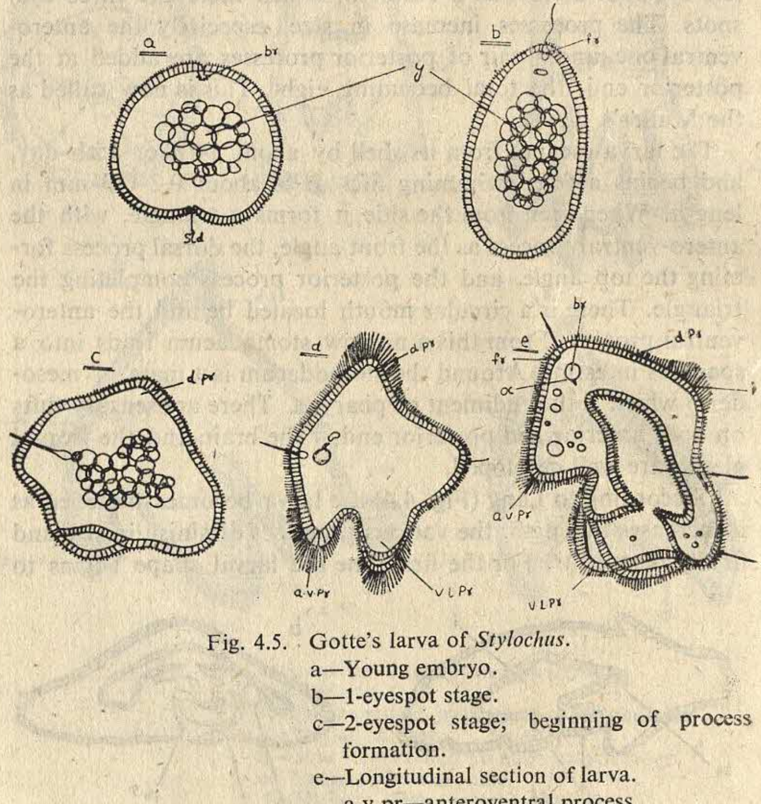


Fig. 4.5. Gotte's larva of *Stylochus*.

a—Young embryo.
b—1-eyespot stage.
c—2-eyespot stage; beginning of process formation.
e—Longitudinal section of larva.
a.v.pr—anteroventral process.
br—brain.
d.pr—dorsal process.
fr—frontal organ.
i—intestine.
o—mouth
oe—eyespot.
std—stomodaeum.
vl.pr—ventro-lateral process.
y—yolk.

the difference being having only four processes instead of eight, these being an anteroventral, a dorsal and a pair of latero-ventrals.

The development of this larva is like that of *Planocera.* The larva measures about 0.1 mm in length, the brain is differentiated near the anterior end of the body, and the frontal gland is not very highly developed. Metamorphosis takes place several days after hatching. Lang believed that this is an intermediate stage of Muller's larva but the rearing experiments of Kato (1940) showed that the Gotte's larva does not acquire eight processes before metamorphosis and hence it is an independent larva.

In the Japanese species, *Planocera reticulata* development proceeds through the Muller's larva stage inside the egg shell and the young animal emerges only after metamorphosis, Kato (1940) called this an intracapsular Muller's larva and this type of development as the intermediate development. This type of larva is flattened, its dorsal process is lacking, the tip of the antero-ventral process is bilobed, the intestine is restricted in size. One Californian species *Graffizoon lobota* measures less than 1 mm in length, closely resembles the Muller's larva, becomes sexually mature. This is considered as a case of Neoteny.

DEVELOPMENT OF SIMPLE EGGS (*Acoela*)

In this group, *Polychoerus caudatas* has been studied by Gardiner (1895) and *Convoluta roscoffensis* by Breslau (1909). The development is of the spiral determinate type and resemble that of the polyclads except that the egg undergoes only one meridional cleavage, giving rise to two blastomeres A and B (Fig. 4.6). The usual four sets of micromeres are given off but as these consist of only two cells each, they are called duets instead of quartets. According to Breslau, the macromeres 3A, 3B are already in the interior, surrounded by the over-lapping micromeres by the time they form the fourth set of micromeres (Fig. 4.6). In both the cases a stereogastrula is formed. The interior is filled up with 4A, 4B and the descendents of 4a and 4b. The blastopore closes and the surface cells separate as epidermis. Internally, a clear distinction becomes established between the cells of endoderm and mesoderm. The

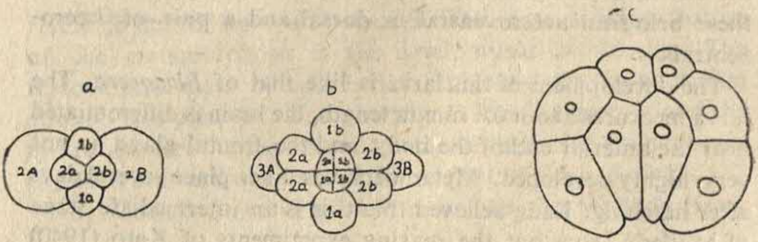


Fig. 4.6. Cleavage in the Acoela.
a—*Polychoerus*—6-cell stage.
b—16-cell stage.
c—*Convoluta* 12-cell stage.

mouth originates by ectodermal invagination near the formal blastopore but no intestinal cavity appears. A free larval stage is lacking and the animal remains permanently in a stereogastrula stage. The nervous system is formed by the first set of micromeres.

DEVELOPMENT OF COMPOSITE EGGS (*Tricladida*)

The development of the triclads was known through the work of Metschnikoff (1883), Ijima (1884), Mallicsen (1904) and Fulinski (1914, 1916). In these species it is common to find within one egg shell (capsule), a large number of yolk cells, sometimes as many as several thousand, surrounding few egg cells. In the planaria there are generally four to six eggs, while in the species *Dendrocoelum lacteum* there are said to be about 20-40 eggs. The cleavage is peculiar. The blastomeres become isolated from each other and distribute themselves among the yolk cells. Under these conditions, it is difficult to find spiral cleavage and regular germ layer formation does not take place. Certain blastomeres flatten to form an outer membrane. This membrane encloses a fluid yolk mass containing the remaining scattered blastomeres. This outer membrane is also called as the provisional ectoderm. At the same time some of the scattered blastomeres aggregate at one point in contact with the outer membrane and there form a temporary embryonic pharynx. A small sac, the temporary intestine is also formed at the inner end of the pharynx. The embryonic pharynx by sacking movements swallows the whole of the yolk syncytium into the temporary intestine. The temporary intestine swells enormously and its wall approaches the outer membrane pushing the remaining blastomeres before it so that a spherical hollow embryo results having a cavity full of yolk cells, an embryonic pharynx and a wall composed of free blastomeres floating in yolk and bounded externally by the original outer membrane, internally by the distended membrane of the temporary intestine. This embryonic wall forms the whole of the future worm.

Within the embryonic wall, the blastomere, suspended in yolk, proliferate actively, and the resulting cells eventually gather at the ventral side, which is determined by the embryonic pharynx and form the germinal cord along the mid-ventral line. This embryonic end consists of three cell masses, the middle one near the embryonic pharynx and the others lying anterior and posterior to it.

The embryo flattens out, and the embryonic pharynx, the inner and outer membranes begin to degenerate. The innermost cells of the embryonic masses correspond to entoderm, and they invade the yolk cells. Eventually they form the intestine at first as a simple sac, later becoming branched by the invasion of mesenchyme at various points. The rest of the embryonic masses represent the ectoderm and the mesenchyme. The anterior mass, because of its close association with the epidermis is largely ectodermal, forms the cerebral ganglia as separate masses that later unite and from this the remaining nervous system is given out. The central embryonic mass produces the pharynx by forming cavities that become the pharyngeal chamber and the lumen of the pharynx. From the different appearance of the cells the linings of these cavities are taken to be ectodermal while the remaining pharyngeal structure is mesenchymal. A definite stomodaeal invagination is absent. The remaining indifferent cells of the embryonic masses are mesenchymal and give rise to the sub-epidermal and parenchymal muscles, the gland cells and other cells of the general mesenchyme, and eventually to the reproductive system.

The embryos hatch in two or three weeks as complete minute worms by the rupture of the capsule often along a predetermined line. In most cases as soon as the young worms have reached a certain size, the reproductive system appears, formed from the free cells of the mesenchyme. Some of these forms small clusters which eventually differentiate into ovaries and testes. The copulatory complex arises from a mass of mesenchyme that aggregates in the appropriate location, hollows out to form the cavities, and differentiates into the lining epithelia, muscle layers and gland cells characteristic of this complex.

DEVELOPMENT OF COMPOSITE EGG (*Rhabdocoela* and *Allocoela*)

The development of several rhabdocoels and allocoels has been investigated by Bresslan (1904), Hallez (1909) and Ball (1916). Usually each egg capsule contains one egg surrounded by several hundred yolk cells. In the species *Plagiostoma girardi* there are 10-12 eggs in a capsule. The number of yolk cells varies widely with the type of the egg. In the family of the Rhabdocoel, Typhloplanae, the genera *Mesostoma*, *Typhloplana*, *Bothromesostoma* etc. lay two types of eggs: small summer (or sabitaneous) eggs with thin shells, and large, thick-shelled winter eggs (also called danerie, latent on dormant eggs). The summer eggs have few yolk cells, whereas winter eggs have many. The species *Mesostoma chrenbergi* is famous for its large size. The winter eggs hatch out in spring and form summer eggs. These eggs are self-fertilized, hatch, and grow to maturity within the parental body, finally coming out by rupturing the parental body. This process occurs repeatedly during the summer, until the reproductive organs finally reach maturity in the autumn and the animals copulate and form winter eggs. At the death of the parent, these eggs are released into the water, where they remain dormant throughout winter.

Development with yolk cells is highly modified, and although traces of spiral cleavage are seen in some cases there is no possibility of following the fate of the various cells and no process, resembling germ layer formation through gastrulation has been seen.

The egg surrounded by yolk cells undergoes total cleavage, the blastomere form into clusters which gather at one side of the yolk. During this time, the yolk cells fuse together and form a syncytium. The side where the blastomere masses collected is the future ventral side of the embryo, while the other half occupied by the yolk will be the dorsal side. The blastomere mass often shows a bilateral arrangement along a plane which is the future median sagital plane, and becomes divided anteroposteriorly into three masses. The germinal cells near the surface of the embryo become arranged in a layer and form the epithelium of the ventral surface. This gradually spreads dorsally to enclose the dorsal yolk mass. The cells on the opposite side i.e. those in contact with the yolk, become endodermal. These digest the yolk and form the intestinal sac. The anterior of the three embryonic masses develops into the paired cerebral ganglia which later become united by commissures and from which the rest of the nervous system grows out. Pigment cells and the retinal cells of the eyes grow out from this brain mass. The middle embryonic mass represents the musculature, glands and parenchyma of the pharynx. The epithelium of the pharynx, the lining of the pharyngeal cavity and the epithelium of the oesophagus come from a solid epidermal in growth that later becomes hollow. The posterior embryonic mass forms the posterior end of the worm and the reproductive system. The intestine and general parenchyma come from those cells of the embryonic masses that are nearest the yolk. Some of these work into and absorb the yolk mass and then aggregate to form the intestine solid at first, later hollowing out. Other indifferent cells of the embryonic masses differentiate into parenchyma and subepidermal and parenchymal musculature.

Comparing the developmental process of these types of eggs, it appears, that while the eggs of Acoela and Polycladida, together with those of the Nemertini undergo typical spiral cleavage, the cleavage patterns of the composite eggs are quite different, and show an extreme degree of modification. The members of Acoela and Polycladida give rise to Muller's, Gott's and Pilidium larvae in their development and undergo a clear-cut process of metamorphosis. In the other groups where composite eggs are present; there is striking metamorphosis and conspicuous variety of forms.

The Monogenea resemble the Rhabdocoela, and genera like *Gyrodactylus* exhibit polyembryony. In Digenea, the life history becomes extremely complicated, exhibits heterogony or alternation of hermaphroditic with parthenogenetic generation with an exchange of host animals. The adults of the sexual generation, which lay fertilized eggs usually have vertebrate animals as their definitive hosts.

The cestodes undergo striking metamorphosis, usually do not reproduce during the larval stages. The embryos called as *lycophora* and *onchosphaera* or *hexacantha* develop into various larvae depending upon their species.

ASEXUAL REPRODUCTION

Asexual reproduction occurs in the rhabdocoel families Catenulidae and Microstomidae, in the fresh water triclads of the family Planaridae and in some land triclads. The rhabdocoels undergo transverse fission into chains of zooids which become well-differentiated before breaking from the chain. Multiplication by transverse fission is of common occurrence among fresh-water planarians. The fission takes place when the conditions are suitable and the animal has attained a certain size. The rear end suddenly attaches firmly while the anterior region continues to advance so that the fission region is pulled out to an elongated shape and quickly breaks. The isolated posterior end soon develops into a small complete worm. Fission can be prevented by arresting locomotion and adhesion. Rise of temperature and decapitation induce fission. Generally fission occurs at a certain level of the body, below the pharynx but it may take place at other levels, or in front of the pharynx in some species.

The work of Child (1911) indicates that the fission plane is predetermined physiologically and that the part of the animal behind this plane already has physiological characteristics of a new individual. In nature asexual reproduction of planarians is generally related to the seasonal cycle. Most commonly fissions occur at frequent intervals during the summer and sexual reproduction takes place in winter and spring.

REGENERATION

Those Turbellaria that reproduce asexually also possess high powers of regeneration. In other Turbellaria regeneration of a head is limited to pieces from anterior regions or to pieces containing the cerebral ganglia. Many forms can regenerate the posterior parts like pharynx and copulatory organs if the cuts are made behind the cerebral ganglia. In all the cases the region in front of the cerebral ganglia is incapable of regeneration. The cut pieces show polarity, i.e. the anterior cut surface regenerates head and the posterior cut surface a tail. The capacity to regenerate, the rate of regeneration, the amount of regenerated material and the type of tissue that is produced depends upon the level of the cut made, it being greatest anteriorly and declines posteriorly.

Regeneration involves two processes; regeneration proper or *epimorphosis*. This means the growth of new tissue from the cut surface in partial replacement of the part removed. The second process is *morphollaxis* or the working over of the old tissue and organs to fit into the new animal. The relative roles of these two processes vary with different species and with size and level of the piece.

The Acoela show very little ability for regeneration.

The rhabdocoels which form chains of zooids regenerate readily pieces from almost any region, reorganise into complete new worms. The rhabdocoels, which does not reproduce asexually, and all the allococoels are incapable of any regeneration. The wounds are however healed by epidermal migration.

Fresh-water planarians of the genera *Dugesia*, *Phagoeata*, *Polyectis* etc. show remarkable powers of regeneration. Pieces of moderate size from any level form a complete new worm. The size, and normality of the regenerated head, the rate of anterior regeneration and the length of tail produced are generally greater at the anterior level and decline at the posterior levels. In general, the ability of a piece to regenerate into a complete worm of typical morphology depends upon the regeneration of a head at the anterior cut surface. This means that the head controls the pattern of the morphology. Very short pieces especially from anterior regions may regenerate biaxial heads i.e. form a head at each cut surface. Isolated heads cut off just behind the eyes regenerate a reversed head at the cut surface. In some species short posterior pieces produce biaxial tails. If oblique cuts are made, a head usually forms at the most anterior point, a tail at the most posterior point.

By means of appropriate splits, double or multiple heads on tails may be induced by will. Regeneration is also affected by external factors such as temperature, osmotic pressure and chemical composition of the medium. If a sexually mature planarian is cut into two between the pharynx and the copulatory apparatus, the latter regenerate, and the piece regenerates into an asexual worm. The large anterior piece regenerates a new copulatory apparatus if active testes are present. The presence of ovaries are not important. This result indicates that the formation and maintenance of copulatory organs is dependent on the presence of testes.

EXPERIMENTAL EMBRYOLOGY

Benazzi (1963) has used X-ray irradiation to investigate the function of sperm in fertilization. He irradiated whole *Dugesia lugubris* with 1,000 to 7,000 r. and allowed the sperms from these animals to fertilize the two types of eggs. These sperms were found to fertilize both the types of eggs. Since X-rays act on the nuclear material, Benazzi pointed out that the two different functions of sperm in fertilization are: (1) activation of the egg, and (2) contribution of nuclear material to the zygote. He also found that the longevity of the irradiated sperms is striking, they can live for several weeks. Seilern-Aspang (1958) centrifuged the cocoons, two to six hours after they are laid. He found out that the sensitivity to centrifugation is clear-cut and corresponds to the time when the yolk-cells are clumping around the egg-cell and ends when the yolk-cell clump is incomplete. It seems likely that centrifugation of cocoons when the yolk-cells are clumping results in symmetrical 'Siamese' twins or triplets by causing two or even three egg-cells to lie close together so that their sphere of influence on the yolk cells overlap. A wide variety of types of Siamese twins is produced by centrifugation at the stage of yolk-cell clumping.

5. *Nemathelminthes*

The normal development of the phylum Nemathelminthes, particularly its order Nematóda have been studied by several workers.

EARLY DEVELOPMENT: SPERMATOZOA

The spermatozoa are amoeboid. In the genus *Parascaris* the spermatozoa consists of two parts, the sperm cell and the acorn-shaped body having cytoplasm. The cytoplasmic part is bigger than the nuclear part and contains small bodies known as plastosomen. These are mitochondrial in nature. The spermatozoa of *Rhabditis ikedai* are oval or triangular. They live within the female seminal receptacle with thin pointed cups turned towards the oviduct. When kept in Ringer's solution, they show active amoeboid movement by putting forth finger-shaped or branching pseudopodia from any point. This helps them to rotate frequently.

OOCYTES

In *Parascaris*, the mature oocytes are triangularly ovoid, with one rounded pole and another pointed. The rounded pole faces the oviduct. As the oocytes pass into the uterus they become spherical. They are covered by a thin semi-transparent membrane on the outside and the cytoplasm contains many granules. Schleip (1929) described these granules as yolk granules. Fauri-Fremiet calls them as lipoid bodies. The immature oocytes have a fixed polarity.

FERTILIZATION

In Nematoda the fertilization is internal, syngamy occurs simultaneously with the formation of a perivitelline space. The further development depends upon many factors like temperature, oxygen etc. In the genus *Rhabditis* the egg changes from spherical to an ovoid structure after fertilization. In *Parascaris*, the spot where sperm has entered becomes hyaline and the granules collect at the centre of the egg. The plasma membrane in the region of sperm entry becomes indistinct. The cytoplasmic granules and vacuoles begin centrifugal and centripetal movements.

The eggs of oviparous species have a thick and resistant egg membrane. In the egg membrane of *Oxyurus vermicularis*, there are four layers called as A, B, C and D from outside. The egg membrane of *Ascaris* depending upon the mode of formation show five layers, the outermost being called as the first layer. The first layer is secreted by the uterine wall, and the remaining layers are formed from egg cytoplasm. Each layer is composed of a basal membrane and an additional membrane. The fifth layer however is different from the others and resembles the 'D' layer of *Oxyurus*. In viviparous and ovo-viviparous individuals, the egg membrane is generally thin.

FORMATION OF THE VARIOUS LAYERS OF EGG MEMBRANE

The first layer is secreted by the uterine wall. A number of granules are first secreted and these swell and fuse together to form the membrane. This membrane is wavy in outline, contains lot of albumin and can be peeled off from the egg. It is absent in viviparous and ovo-viviparous individuals.

The second layer starts its existence after the entry of the sperm. The large granules of the cytoplasm migrate from the centre of the egg to the periphery and in this process form vacuoles. The medium-sized granules of the cytoplasm adhere round the vacuoles and join together to form a lipoid layer. This adheres to the inner side of the plasma membrane forming the second layer. At first the layer is irregular, but during the formation of the third layer it becomes smooth.

As soon as the second layer has been formed the small granules of the cytoplasm move from the centre to the periphery. These swell and with a hyaline substance become attached to the inner surface of the second layer forming a thin basal membrane. The additional membrane is formed in the same way as the first membrane is formed.

In the same way as in the third layer, in the fourth layer also a basal membrane is formed. Then by the movement to the periphery and vacuolization of large and small granules, the additional layer of the fourth membrane is formed and attached to the basal membrane. The vacuoles as they reach the periphery become very large and lie on top of one another giving the effect of soap bubbles. The large vacuoles also break down and merge their contents with those of the vacuoles. This layer becomes smooth when the fifth layer is formed. It resembles the second layer except it is lustrous and strong. At this stage, the number of cytoplasmic granules decrease, the interior of the cell becomes brighter and granules change from a yellowish-black to brownish-yellow.

The oocyte nucleus along with the granules moves towards the periphery and there gives off the first polar body. At this stage the fifth layer of the egg membrane is formed. Because of this the first polar body becomes flattened and present beneath the fourth layer.

After the first polar body is given off the oocyte nucleus moves back to the centre. A homogenous layer appears towards the periphery. Small granules and swollen vacuoles move into this area fusing and dissolving to give rise to very delicate lamellae. In this process the granules break at a slower rate than the vacuoles. Due to this activity there is a gradual increase in the fibrous material on the inner side of the fourth layer. At the end of this process a rough and thick fifth layer is formed. This becomes thin and dense by the formation of the perivitelline space.

After the formation of the fifth layer, a clear protoplasmic area with small granules appears in the egg cortex. These granules move outward and become arranged in the clear cytoplasm. The granules swell, fuse to form the plasma membrane. This membrane is irregular at first and then becomes smooth as the perivitelline space and external cytoplasmic zone are formed.

A thin hyaline membrane also appears outside the plasma membrane from the same granules which help in the formation of the plasma membrane, certain swollen vacuoles extrude through the hyaline membrane and spread out between it and the inner surface of the fifth layer to form the perivitelline space. At this stage, the hyaline and the plasma membranes become thinner and denser. After the formation of the perivitelline space the egg becomes transparent.

At about the same time when the perivitelline space is formed, the second polar body is given out. The oocyte nucleus returns to the centre of the egg. The sperm pronucleus and the egg pronucleus unites and granules gather around the fused nucleus. The mature eggs of most nematodes are ovoid but different shapes also occur among the various genera. Usually the egg membrane is smooth but some have processes or pits. In some forms there are lid-like structures or plugs at the two poles of the shell.

CLEAVAGE AND GERM LAYER FORMATION

In the case of *Parascaris*, the cleavage is total and equal. The first cleavage plane is perpendicular to the polar axis, dividing the egg into animal pole side S_1 and vegetal pole side P_1 blastomere. The separation into somatic and germ cell lines takes place from the two-cell stage by a phenomenon called chromatic diminution. One cleavage cycle may be divided into two phases. The first phase includes the time from the appearance of the mitotic figure to the completion of nuclear division and the formation of the cleavage furrow. In this phase the position of the cleavage plane is determined and its basic area is established. The second phase includes the time covered by the new formation of the cleavage plane and the completion of the daughter nuclei. The cleavage surfaces are newly formed with hyaline protoplasm and mitochondrial granules. The migration and swelling of the granules also take place at this stage. The apposed surface of the blastomeres constitute the new surface and these are flattened against each other.

Immediately after the maturation, the egg of *Parascaris* is fairly oval, the peripheral zone is made up of pale yellowish green hyaline substance (Fig. 5.1). The interior protoplasm is crowded with a large number of variously sized granules and also large vacuoles scattered here and there. Granules of all sizes change to vacuoles and then dissolve into this hyaline zone. As a result of this the contrast between the external and internal cytoplasm is heightened. Then the egg becomes spherical (Fig. 5.1 A).

The egg and sperm pronuclei make contact in the centre of the egg and fuse forming the transparent fusion nucleus in which the chromosomes are clearly visible. The sphere of fusion nucleus gradually expands into a spindle shape. The spindle fibres become visible and asters develop at both ends. A little outside the spindle, a spindle-shaped boundary profile can be differentiated from the endoplasm.

The mitotic figure moves with the internal cytoplasm to the vegetal pole and there is less peripheral cytoplasm at the vegetal than at the animal pole (Fig. 5.1 B). When the mitotic figure moves back to the central region, there is a return of peripheral plasm to the vegetal pole and the formation of new plasm takes place so that the peripheral layer becomes uniform around the egg. This layer is called as the peripheral cytoplasm (Fig. 5.1 C).

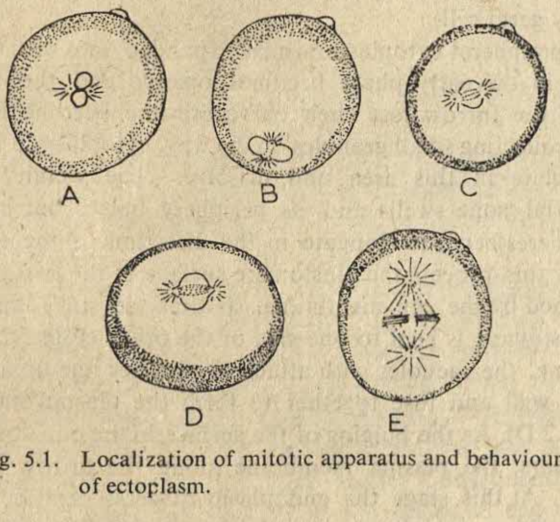


Fig. 5.1. Localization of mitotic apparatus and behaviour of ectoplasm.

As the mitotic figure develops it moves towards the periphery at the animal pole and the egg takes an asymmetrically oval shape (Fig. 5.1 D). At this time the peripheral plasm is more at the vegetal side and less at the animal pole. Turning through 90°, the mitotic spindle elongates further and as it reaches the centre of the egg, the mitotic process reaches the metaphase. At this time there is more of peripheral plasm at the animal pole and less at the other regions (Fig. 5.1 E). After the rotation of the mitotic figure, the distance between the centre of the asters and the egg surface is greater at the animal pole side. With the elongation of the spindle the whole egg elongates and the cleavage furrow is formed. As the cleavage furrow advances centrally from the outside of the equatorial zone, a furrow forms cutting the nuclear region also (Fig. 5.2) and finally the two blastomeres are connected with each other at only one point (Fig. 5.2 A). The egg is cut into a larger S_1 blastomere containing more of the peripheral cytoplasm and a smaller P_1 blastomere which has more endoplasm. The blastomeres which derive from S_1 are the somatic cells and P_1 gives rise to the primary germ cell.

The peripheral cytoplasm which expanded into the cleavage furrow in the early phase becomes opaque and the apposed face of the furrow lose their curvature and become flat (Fig. 5.2 B) collecting small granules on the way, cytoplasmic vacuoles accumulate in this area and dissolve. The vicinity of the equatorial zone swells and its periphery bulges out and both blastomeres begin to elongate in the direction of the egg axis. During this process the blastomere surface at the two poles are restrained by the egg membrane, so that the stalk connecting the blastomere is bent to one side or the other (Fig. 5.2 C). At this time, the vacuoles with attached granules line up along the furrow wall and fuse together to form the plasma membrane (Fig. 5.2 D). As the bulging of the surface in the equatorial zone disappears, the plasma membrane along the furrow becomes thicker. At this stage the endoplasm disperses and a layer of homogeneous peripheral cytoplasm appears around all the surfaces of the blastomeres except for the apposed furrow faces where no such zone is seen (Fig. 5.2 E). The nuclear region, which was divided between the two blastomeres forms at first two pear-shaped masses (Fig. 5.2 F) but small mitochondria-like granules from the endoplasm surround these and break-

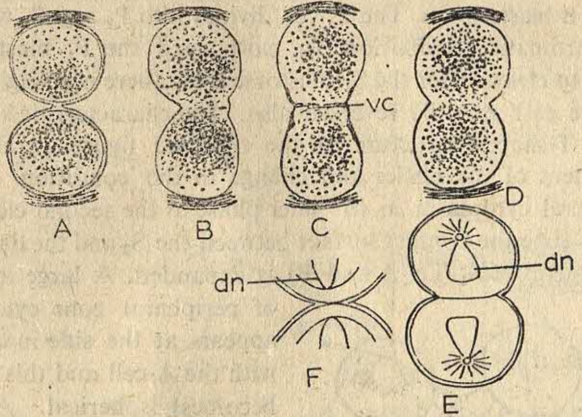


Fig. 5.2. Cleavage of *Ascaris*.
A, C—First process of cleavage.
B, D, E, F—Second process of cleavage.
Vc—vacuole.
dn—daughter nucleus.

down resulting in spherical daughter nuclei with thick nuclear membranes.

In the eggs of *Rhabditis ikedai* and *Rh.* sp. the furrow of the early phase cuts simultaneously into the equatorial zone from all sides but stops, leaving one-third of the diameter uncleaved. This roughly corresponds to the nuclear region. The spindle fibres remain uncleaved. With the disappearance of the spindle, granules appear at the equatorial region and the nuclear region is divided into two. These unite with swollen granules which have migrated into the equatorial region and form the walls of the furrow. These eggs do not show the bulging of the periphery in the equatorial zone on the polar elongation that was seen in the *Parascaris* egg during the later phase. Consequently the early and later phase of cleavage in the *Rhabditis* egg differs from those of *Parascaris*.

The second cleavage plane is parallel to the egg axis in the S_1 blastomere and perpendicular in P_1 blastomere, producing a T-shaped four-cell stage which later turns into a rhombic-shaped structure. This is achieved by the shifting of the position of the P_2 blastomere (Fig. 5.3). The chromosomes of the S_1 blastomere are cut off and thrown out into the cytoplasm. With the diminution of the chromatic material, the S_1 blastomere divide into A and B blastomeres. The P_1 cell divides into P_2 and S_2 without any chromatic diminution. In most cases the P_1 blastomere begins to cleave after the S_1 blastomere but there is no rigid rule, and the case may be reversed also. The characteristic shifting of the T-shaped structure to the rhombic figure parallels a movement of the nuclei and change in the condition of the peripheral cytoplasm in the later phase of the second cleavage. At this time the contact surface between the S_2 and the daughter blastomeres of S_1 (i.e. A and B) is expanded. A large amount of peripheral zone cytoplasm appears at the side in contact with the A-cell and this region becomes spherical. At the opposite end i.e. at the B-cell side in the later phase of the P_1 blastomere cleavage, P_1 buds around and approaches the S blastomere and finally B and P_2 unite side by side giving the embryo a rhomboidal shape (Fig. 5.3 C). In this process a profound change occurs in the state of ecto and endoplasm.

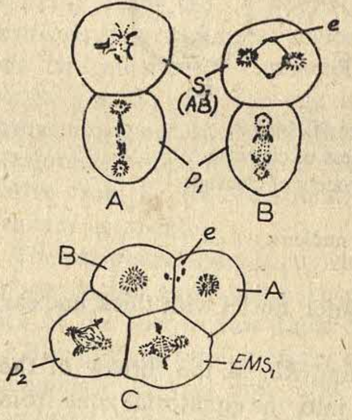


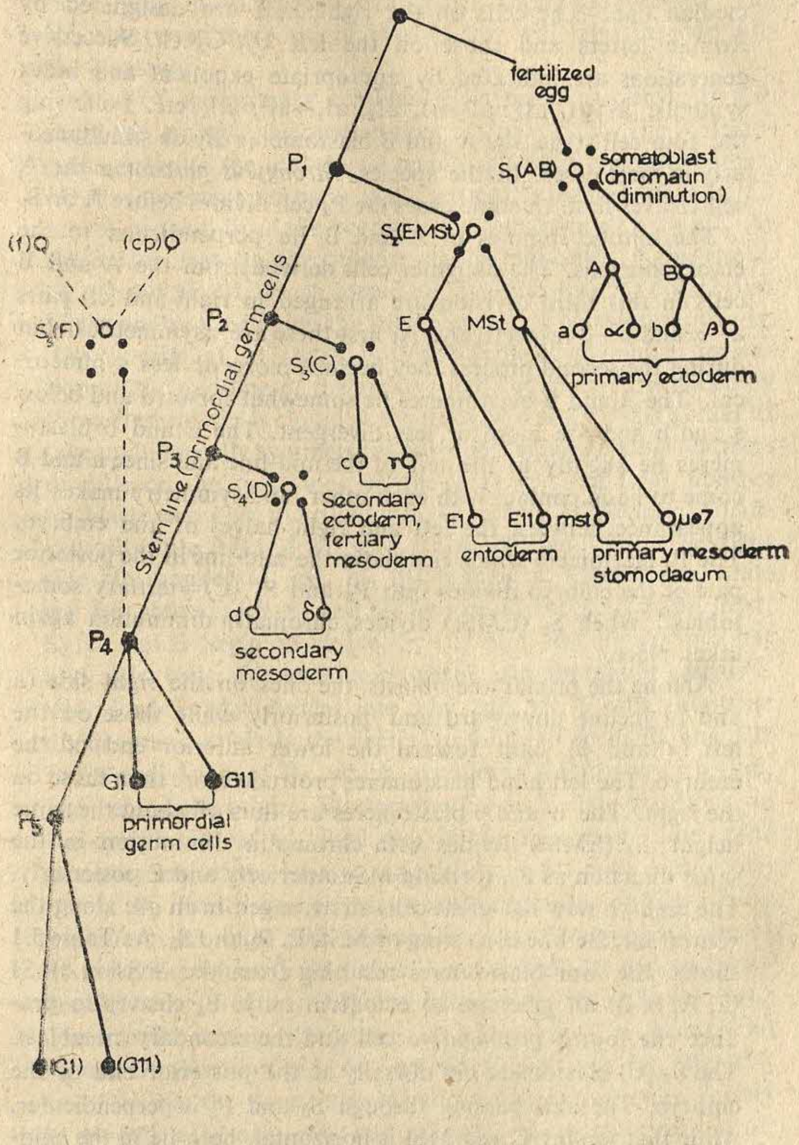
Fig. 5.3. Chromatin diminution in egg of *Ascaris*
A—Metaphase of second cleavage,
B—Anaphase of second cleavage,
C—Metaphase of third cleavage,
e—Eliminated chromation.

As shown in Table 5.1 the A and B blastomeres are the primary somatoblasts. The A and B blastomeres may be of the same size or A may be slightly larger than B, but B contains slightly more endoplasm than A, B_2 is the secondary somatoblast and P_2 is the secondary propagative cell. S_2 is larger than P_2, has more peripheral cytoplasm but less than A and B cells. It has also the largest amount of endoplasm. In the case of *Rh.* sp. the T-shaped figures of the blastomere is not well marked. The amount of peripheral cytoplasm is less than that of *Ascaris* eggs. In the species *Rh. ikedai* the T-shaped larva makes its appearance.

The somatic cells produced by repeated cleavage are designat-

Table 5.1

Cell-lineage in *Parascaris eouorum*



ed by S_1, S_2 etc. and furthermore S_1=AB; S_2=EMSt, S_3=C, S_4=D, S_5=F. A, B, C, D, E, F, M and St are all located in the median plane of the embryo. These cells divide and the resulting cells lie symmetrically to the left and right of the median line. The cells on the right half are designated by Roman letters and those on the left by Greek. Successive generations are indicated by appropriate exponent and index symbols; a→aI, aII, aI→aI, aI_2; aI,→al^1, al^1, etc. Following the four cell stage, the A and B blastomeres divide simultaneously in most cases. In the species, *Strongylus paradoxus* the A cell cleaves first. In some cases the P_2 cell divides before A or B.

The mitotic spindles of A and B lie perpendicular to the embryonic axis. The daughter cells derived from the A and B cells in this third division are arranged in right and left pairs a—α and b—β respectively. At first these are asymmetrical but during the second process they become more or less symmetrical. The A and B blastomeres lie somewhat forward and below a and b and are more or less divergent. The a and b blastomeres lie slightly to the left of the mid-line and since a and B come to lie in contact with each other, an asymmetry makes its appearance between the left and right halves of the embryo. The P_2 blastomere which lies above the mid-line in the posterior part of the embryo divides into P_3 and S_3 (C)=tertiary somatoblast. When S_2 (EMSt) divides, chromatin diminution again takes place.

Among the primary ectoblasts, the ones on the right side (a and b) incline downward and posteriorly while those on the left (α and β) slant toward the lower anterior end of the embryo. The left hand blastomeres protrude more than those on the right. The b and β blastomeres are thus of about the same height. S_2 (EMSt) divides with chromatin diminution in the same direction as P_2, forming MSt anteriorly and E posteriorly. The embryo now has eight cells all arranged in an arc along the ventral middle line consisting of MSt, E, P_3 and S_3. As Table 5.1 shows, the four blastomeres resulting from the division of SI (a, A, b, B) all give rise to ectoderm only. P_3 cleaves to produce the fourth propagative cell and the secondary mesoblast. The S_3 (C) blastomere lies dorsally at the posterior end of the embryo. The axis passing through S_3 and P_3 is perpendicular, while that joining E and MSt is horizontal, both lie in the mid-plane and at right angles to each other. After the division of S_2, α and β are pushed out in a downward direction, a and S_3 (C) come into contact with each other, Mst lies at the front end and S_3 (C) at the back end, and a, α, E and P_3 are continuous to these lying in an annular arrangement along the mid-line. The space between these sets of blastomeres will later develop into the blastocoel.

Some of the primary ectoblast cells begin to cleave; in this stage the embryo becomes rather asymmetrical. The β blastomeres move to the mid-dorsal region and away from each other, aII fits into the upper parts of the space thus formed, while S_3 (C) moves around from the posterior end into contact with aII. At the fourth cleavage, the primary ectodermal cells a, α give rise to aI, aII and βI, βII, which spread out over the anterior dorsal part of the embryo, bringing the total number of blastomeres to ten. The six cells, aI, aII, MSt, E, C and P_3 lie in an annular formation on the mid-line of the embryo and the space surrounded by these blastomeres is closed at the sides by b and β. Next aI and αI take up bilaterally symmetrical positions, equalizing the numbers of cells on the two sides of the embryo. The total number of blastomeres is increased to 12 when b divides dorsoventrally into bI and bII and β into βI and βII. The right side blastomeres aI, aII, bI, bII assume a T-formation, while the left side αI, αII, βI and βII line up in rhombic shape. Mst, S_3 (C) and E begin to cleave. S_3 (C) and Mst divide into left and right cells. S_3, undergoing chromatin diminution, forms C and γ, while Mst divides into Mst and μδt. E, the endoblast divides transversely in the ventral mid-line to form EI and EII but the division of P_3, which also lies in this posterior region, lags behind the others. Blastomeres C and Y take positions on the dorsal side of P_3 which lies in the mid-ventral line at the posterior end of the embryo, and bII and βII face each other. Before P_3 divides the primary ectoderm-producing blastomeres begin to undergo the fifth cleavage, i.e. the aI and αI blastomeres divide dorsoventrally into aI, aI_2 and αI_1, αI_2, bI divides transversely to give bI and bI_2 and bII divides into bII_1 and bII_2. These cells arrange themselves in the form of a rhomboidal figure. On the other side βI and βII give rise to βI_1, βI_2, βII_1 and βII_2. On the dorsal side aII and αII also divide transversely to produce aII_1, aII_2, αII_1, and αII_2 and the daughter cells of aI, aII, αI, αII, bI, bII, βI and βII assume a symmetrical arrangement. In this fifth cleavage the primary ectoderm-producing blastomeres thus give rise to 16 cells.

As the two daughter ventral blastomeres Mst and μσ migrate from their position in front of daughter cells of aI and αI to the ventral side of these blastomeres, they come to lie to the right and left of EI. Then P_3 which lies mid-ventrally divides forming S_4 (D) and P_4. The S_4 lies at the posterior extremity and P_4 on the ventral side of the embryo. The S_4 produces the quaternary somatic cell line, gives rise to the secondary mesoblast. EI, EII, P_4 and D_1, which are derived from the P_1 blastomere lie on the mid-ventral line of the embryo, while Mst and μδt lie side by side at the anterior end and cy are at the posterior end. These ventral blastomeres form an arc which curves dorsally. Next the primary ectoderm forming line of blastomeres carry out the sixth cleavage. During this process the Mst and μδt migrate into a position between EI, EII and the daughter cells formed by bII and βII. As a result of this shuffling Mst and μδt become separated from aI_1 and αI_1.

After this the mst divides transversely into an anterior st and a posterior m blastomere. On the left side μσt gives rise to σt simultaneously and these assume symmetrical positions. Strassen believed that all these blastomeres are primordial mesoderm cells. Boveri thought that only m, μ are mesodermal the st, δt blastomeres are ectodermal in nature and destined to form the stomodaeum. Because of this he called them as stomatoblasts.

At this stage the embryo has become a blastula with a small blastocoel. The endoblast cells EI and EII in the mid-ventral line divide laterally into eI, eII, εI and εII. The two blastomeres at the right side move forward, giving rise to a rhomboidal figure to these four cells and the embryo as a whole loses its spherical form and elongates in an anteroposterior direction. The c blastomere undergoes chromatin diminution and divides obliquely into cI, cII while y divides into yI, yII. These blastomeres are secondary ectoblast and tertiary mesoblast cells. From this stage, the endoblasts become gradually enveloped dorsally and laterally in primary ectoblast cells, while the descendants of P_4 cover the posterior ventral side. From the two sides of the embryo the primary mesoblast cells, particularly the stomatoblasts, approach the mid-ventral line and gastrulation begins. The stomatoblasts divide transversely forming stI, stII, σtI and σtII, and m and μ also divide in the same direction. As, m, μ begin to divide, the secondary mesoblast S_4 (D), which lies posterior to P_4, undergoes chromatic diminution and divides, giving rise to the bilateral pair of blastomeres d and δ. The stomatoblasts also divide slightly ahead of the mesoblasts and all move toward the mid-line, gradually causing the endodermal blastomeres eII, eI, εI and εII to slip into the blastocoel. Eventually the mesodermal cells follow them and the increase in number of blastomeres causes a reduction in the size of the blastopore. On the ventral side of the embryo, a ring is formed around the blastopore, consisting anteriorly and laterally of the blastomeres mI, mII, stI, sTII, uI, uII, δyI and δyII and posteriorly of d, δ and P_4. This P_4 blastomere moves to a position below the endoderm cells while the ectodermal cells shift toward the ventral side and the stomatoblasts stI, stII, δyI and δyII approach still more closely to the mid-line. The ectodermal cells then surround the stomatoblasts from the lower anterior part of the embryo. The primary and secondary mesoblasts (mI, mII, μI, μII) lying alongside and behind the blastopore become surrounded by ectoderm, making the blastopore opening still smaller. In this process, the stomatoblasts stI, stII, δyI, δyII do not invaginate together with the mesodermal blastomeres mI, mII, μI, μII. The descendants of S_3 (C) increase to eight cells and occupy the posterior part of the embryo.

The four blastomeres cII$_1$, cII$_2$ yII$_1$ and yII$_2$, form a bilaterally symmetrical line. These blastomeres are also called ventral cells. In the posterior dorsal region, cI and yI cleave transversely to produce cI$_1$, cI$_2$, yI$_1$, yI$_2$ and these cells envelope the posterior end of the embryo. As these mesodermal blastomeres invaginate, the mesoblasts and stomatoblasts from the S_2 (MESt) blastomere are undergoing division.

Next, the secondary mesoblast cells d, δ on the ventral posterior side divide into dI, dII, δI, δII; these surround the P_4 blastomere and become arranged in a wide arc in the anterior part of the embryo, while P_4 shifts to a position directly below the endoderm cells in the mid-ventral line.

The P_4 cell divides transversely to produce the primordial germ cells which are called as GI and GII. A connection is established between the st, δt and δ blastomeres and the primary mesoblast cells m, μ invaginate. With the migration of the blastomeres at the posterior end of the embryo, the GI and GII are carried inside the blastopore. Then the secondary mesoderm cells invaginate and the ectoderm cells move to the sides of the embryo. The number of stomatoblasts increase to eight (stI_1, stI_2, $stII_1$, $stII_2$, δtI_1, δtI_2, δtII_1, δtII_2) and stI_2 and δtI_2 come into contact with each other. Next, the posterior stomatoblasts establish contact with the secondary ectoblast cells on the posterior ventral side of the embryo and GI and GII, lying at the blastopore are enveloped in ectoderm. At this time the mesodermal cells separate from the ventral cell layer and become completely invaginated. The four endoblasts inside the embryo and these primary mesoblasts proceed to divide and the latter form the lateral embryonic body wall.

After this the stomatoblasts produce a depression and the formation of the stomodaeum begins. When this is going on, the secondary ectoblast cells spread over the posterior ventral surface of the embryo, and the GI and GII blastomeres slip out of the ventral cells, in the blastopore region cells bulge inward, forming a stomodaeal pocket anterior to the germ cells GI and GII. GI and GII are pushed still closer to the endoblasts, ectoderm cells proliferate from the sides and the posterior part of the embryo, and spreading over GI and GII come together in the mid-ventral line. The germ cells separate from the ectoderm and move inward, the anterior cell (GI) penetrating further into the gut rudiment than the other (GII). The stomatoblasts establish connection with the ectoblasts. In this process the blastopore is completely closed off, and posterior to it, the two germ cells are surrounded by mesoderm and endoderm. The ventral ectoderm acquires a smooth outline. In addition to the stomatoblasts, some ectoderm cells also take part in the formation of the stomodaeum (Boveri, 1899). By this process the stomodaeal invagination pushes inward, leaving a small opening and slanting somewhat ventrally, this will later move forward and become the larval mouth. Seen from the side, the stomodaeum becomes shortened, and elliptical in cross section. In the interior of the embryo the endoderm develops into a cell mass, in contact anteriorly with the stomodaeum and is flanked by bands of mesoderm. At the ventral side of the endodermal cell mass are situated the two germ cells.

The various problems associated with the above processes have received somewhat varied interpretations from workers investigating such nematodes as *Ascaris*, *Cucullanus*, *Bradynema*, *Rhabditis* and *Anguillula* and accordingly different names have been used to designate the blastomeres. In *Auguillula aceti* and *Parascaris equorum.*, the first two blastomeres have been called anterior and posterior. In *Ascaris* and *Rhabditis* the dorso-ventral axis is not fixed at the two-cell stage; so the terms animal and vegetal are used. According to Schdeip (1924), the primary egg axis is determined before sperm entrance, Strassen (1906) believed that it becomes fixed between fertilization and the first cleavage. Boveri (1910) felt that it becomes evident when the polar bodies are extruded. Pai (1928) held that the place of sperm entrance becomes the anterior part of the larva. In *Parascaris* and *Rhabditis*, the morphological difference in the cytoplasm at the animal and vegetal poles becomes progressively apparent during the period between polar body extrusion and syngamy and is quite clear by the time syngamy is complete. The dorso-ventral and antero-posterior axis of the larva as well as the median plane are roughly indicated after the two-cell stage, when the embryo shifts from the T to the rhombic-shape; the median plane is the plane which includes the diameters of all four blastomeres.

In *Parascaris* the fate of the blastomeres is as follows, S_1 blastomeres forms most of the epidermis (ectoderm). The primary mesoblast M (Mes. I) forms most of the mesoderm. The E blastomere gives rise to the endoderm. St forms the stomodaeal rudiment, S_3 (C) supplies the primary endoblast cells and forms mesoderm. There are still some unsolved questions regarding the division of P_4 blastomere. According to Boveri (1899) P_4 divides into P_5 and S_5, P_5 then divides into GI and GII, while S_4 forms the f and . . ϕ . . blastomeres at the posterior end of the embryo. Muller (1903), Zoja (1895) and Strassen (1896) considered that GI and GII are derived from P_4. The same thing is also seen in other nematode species like *Cucullanus elegans* (Martini, 1903), *Strargylus paradoxees* (Spemann 1895), *Rh. migrovenosa* (Ziegler, 1896). Boveri (1910) later maintained that the production of P_4 is normal in *Paras-*

caris, but that P_5 and S_5 (F) may be formed as an exception. According to Pai, the P_5 blastomere always appears in *Anguillula aceti*, as well as the five groups of somatic cells, the S_1 blastomere giving rise to the primary ectoderm cells, S_2 (EMSt) to primary and secondary endoderm and mesoderm, S_3 and S_4 to the secondary and tertiary ectoderm, S_5 to tertiary endoderm, P_5 to the primordial germ cells. The S_5 blastomere produces the gonad tissue. In *Parascaris*, it is not clear whether S_4 is responsible for forming the gonad. Strassen discovered two cells (Terminalzelle) lying anterior and posterior to GI and GII after gastrulation. He believed that these cells participate in the formation of the gonad. These terminal cells can also be recognised in *Rhabditis*, but it is not known whether they are derived from S_4 or S_5. According to Boveri, the P_3 blastomere is produced at the 82-cell stage; Strassen says it is formed at the 51-cell stage and Martin believes it is formed at the 52-cell stage in *Cucullanus elegans*.

Several opinions have also been expressed with respect to the mode of gastrula formation in *Parascaris*. Strassen believed it to take place by invagination, while Boveri held that the absence of an archenteron points to epiboly. The observations of Hallez (1885, 1887) and other workers also led to results agreeing with Boveri's conclusion. However, Seuret (1920) and Martini (1907) formulated the following modes of gastrulation in various nematodes; (a) conjunctive emboly: In this the invagination is by emboly in which an archenteron is formed to some extent e.g. *Rh. buformis*; (b) disjunctive epiboly: In this the cells are released into the blastocoel as the result of epiboly e.g. *Parascaris equorum*; (c) conjunctive epiboly: Here the gastrulation is intermediate between the above two e.g. *Camallanus lacustris*, *Pseudalius minor* and *Cosmocerea onnata*.

In the early development of *Parascaris equorum* there are differences among the types of cytoplasm of the cell groups which form each of the primary germ layers. The endodermic cells contain more endoplasm and the cells which are destined to form ectoderm have more ectoplasm.

In *Rh. nigrovenosa* and *Rh. ikedai* the endoderm starts invaginating when the descendant of the S_1 blastomere reach a number of 32-cells, at this stage, the total number of cells is about 60. When there are about 180 cells the blastopore closes. In the genus *Anguillula*, gastrula is formed by 141 cells stage, by this time the S1 has divided into 115-cells. Before the gastrula stage, the arrangement of the blastomeres is somewhat asymmetrical, but after gastrula formation, they become symmetrical. The time between syngamy and gastrulation varies from species to species and also depends upon temperature.

Although nematode development as described above differs in certain respects from that of *Parascaris*, it agrees in essence with the reports of Boveri (1887, 1899, 1910) and Strassen (1896), and the research of these two men forms the foundation of nematode embryology.

CHROMATIN DIMINUTION

The phenomenon in which part of the chromatin is discarded into the cytoplasm was first discovered in *Parascaris equorum* by Boveri (1877) and it was also he who made the later detailed observations of the process (1890, 1892). His work was repeated and confirmed by Dostolewsky (1888), Schneider (1891), Herla (1893). Boveri (1899) adapted the term diminution as used by Herla, to describe this phenomenon, calling it 'chromatin diminution'.

As has been stated above, nematode cleavage proceeds in a determinative fashion, and the differentiation of the somatic cell line from the germ cell line begins as early as the two-cell stage. The germ line cells preserve their chromatosomes intact through the division process, but the somatic cells cast off material from the ends of their chromosomes into the cytoplasm between metaphase and anaphase of each nuclear division, reducing the amount of chromatin left in the nucleus. This behaviour makes it possible to distinguish between the germ cells and the somatic cells making up all the other organs of the body. If we consider the two blastomeres P_1 and S_1 resulting from the first cleavage, we see that P_1 possesses the capacity to form nearly the whole embryo. It can therefore be called as universal, while the S_1 blastomere by contrast may be called 'partial'. In *Parascaris equorum*, the ends of the S_1 (AB) chromosomes thicken into a flask-shape by the metaphase and during the period between metaphase and telophase they are cast out of the nuclear region into the cytoplasm where they lie scattered, in a row or chain or all clumped at one side.

The central part of the chromosomal rod becomes a series of particles connected by a hyaline matrix. The particles then separate into granules, the hyaline matrix disappears, and the granules are distributed to the two new blastomeres to form the daughter nuclei. According to Boveri there are about 60 of these granules. In the newly formed blastopore the daughter nucleus appears, with the granules clustered in its center and the discarded chromatin lies scattered in the cytoplasm around it. One opinion holds that these flask-shaped portions of the S_1 chromosome become recognizable only after syngamy and do not contain any genes. While these end portions are strongly stainable with gention violet, they closely resemble the P_1 chromosomes, but they lose their stainability somewhat when the central part breaks up into separate particles. According to T.P. Lin (1954), the discarded material is heterochromatin, and contains large amount of DNA. Van Benedu et Neyt (1887) reported that at discarded material is heterochromatin and contains large the mitotic metaphase these portions of the chromosomes which are to be discarded either fail to develop spindle fibres, or develop them poorly, *Parascaris equorum* includes the two varieties, *univalens*, in which n=2. However, each of these units may be thought of as a compound chromosome composed of a large number of small granular chromatosomes. According to Geinitz (1915) *Parascaris equorum* var. *univalens* has a single large compound chromosome composed of an x-complex made up of eight components, and an antosomic complex containing 22 components. Since *P. equorum* var. *bivalens* discards a large amount of chromatin than univalens, the somatic resting nucleic of the two varieties after diminution are about the same size. On the other hand, the resting nuclei of the germ line cells (P 1-4) and of the primordial germ cells (GI and GII) are clearly larger in var. *bivalens*. Moreover, the vesicular resting nuclei of the somatic cells after chromosome diminution are oval or spherical, while the resting nuclei of the germ line cells characteristically have a number of pouch-shaped processes (Fig. 5.3) equal to the number of ends of the chromosomes contained in them. This chromatin diminution phenomenon takes place during each cleavage of the somatic line blastomere from S_1 to S_4 or S_5, so that only the germ cells descended from the P_4 (or P_2 blastomere retain a set of complete chromosomes).

Strassen (1895, 1896) believed the cause of chromatin diminution to depend on some essential factor located in the nucleus. Boveri (1910 a, b) found that chromatin diminution takes place only in the animal pole centrifuged eggs. He considered that the cause for the chromatin diminution lies in the cytoplasm either at the animal pole or vegetal pole. Beams (1937, 1938) stated that the chromatin diminution is due to 'chemical diminisher' originating in the cytoplasm, with a distribution gradient along the uncleaved egg axis, decreasing toward the animal pole.

It may be possible by using chemical reagents to bring about a reversible conversion of germ line blastomeres into somatic blastomeres and vice-versa. In eggs treated with Liel, the germ line blastomeres are induced to undergo chromatin diminution and in size, rate of cleavage, endo-ectoplasmic relation, these blastomeres resemble normal somatic line blastomeres.

In many nematodes the various organs are characterized by cell constancy as shown by the studies of Martin (1906, 1907) on *Oxyuris*, Goldschmidt (1908, 1909, 1910) on *Parascaris* and Pai (1928) on *Anguillula*. In other words, that every cell in the nematode body has a definite shape, size and position and that the number of cells is determined by the time moulting is complete, with no changes occurring thereafter, except that the boundaries between cells may disappear to some extent after ecdysis, making it difficult to confirm the cell constancy of such tissues as the epithelium in any but the small nematodes.

After the gastrula stage the embryo loses its oval form, the anterior end becomes thicker while the posterior end becomes thinner and bends antero-ventrally performing localized movements. As a result of extremely accelerated growth of the dorsal ectoderm, the part of the ventral surface and the ectoderm of the posterior end also develops and thickens. Next the anterior end curves ventrally bringing the dorsal part into contact with the inside of the egg membrane. As it grows inside the membrane, the embryo takes on a spiral or S shape, when the membrane is thin, as in *Metastrongylus elongatus* or *Rh. ikedai* its shape changes as the embryo elongates.

The cells making up the epidermis in the central part of the curved embryo are arranged in longitudinal rows, forming regular layers and sections. In *Anguillula* according to Pai, the S_1 blastomere gives rise to about four-fifth of the epithelium and S_3 (C) to about one-fifth. In *Cucullanus* the cells form two rows each on the ventral and lateral surfaces and one row dorsally consisting of 11, 12 and 24 cells. These form the entire epidermis extending from anterior to posterior end. The cells give rise to three-quarters of the cells and the S_3 blastomere to one-fourth. The cells derived from the S_3 blastomere cover the posterior-dorsal surface of the embryo. In *P. equorum* according to Boveri at least 12 to 14 cells derived from S_3 (E) blastomere take part in the formation of the epidermis. As growth proceeds the epidermal cells at the anterior and posterior ends of the embryo become flattened and lose their regular alignment. A tough surface layer called as cuticula and an underlying sub-cuticular layer are formed. Even at the initial stages the cell boundaries in the cuticular region are indistinct and as growth proceeds, they completely disappear, giving rise to a syncytium. The sub-cuticular cells become arranged in a linear series along the lateral, dorsal and ventral surfaces. In some places they retain the regular distribution of the early stages.

ORGANOGENESIS

Strassen believed that the stomodaeum is formed by the cells derived from the stomatoblast St; Boveri thought that besides St the blastomere S_1 (AB) also contributed towards the formation of the stomodaeum. Martini held the view that St formed the muscular part of the stomodaeum and the other parts are formed by S_2 blastomere. In *Anguillula*, the pharynx is composed of 59 cells while the oesophagus consists of five cells (Pai, 1928).

The E blastomere which gets separated from S_2 (EMSt) gives rise to 12 large endoderm cells and these cells form the rudiment of the midgut. These cells become arranged in two rows. The midguts of *Cucullanus elegans* and *Rh. migrovenosa* are made up of 16 cells, and that of *Anguillula* of 18. In general the small nematodes have smaller number of cells. As the embryo grows, these longitudinally arranged cells form a simple tube. The lumen of this tube at the beginning is restricted but later widens. This tube gets connected with the foregut anteriorly and with midgut posteriorly. Next the lumen of the foregut and midgut join and a constriction is formed at this region since the midgut of *Anguillula* has no glands, Pai considered it to have an absorbing function. According to Martini and Pai, the midgut is derived from the S_4 (D) blastomere. In *Anguillula*, it is formed of 20 cells which are said to make contact with an infolding of the ectoderm in the posterior part of the embryo. The hindgut of male connects with the ejaculatory duct and opens into the cloaca.

The central nervous system in the ascarids is derived from the S_1 (AB) blastomere, when the stomodaeum is formed, the surrounding S_1 line blastomeres enlarge, proliferate toward the interior and form the nerve ring. In *Anguillula* the cells descended from S_1 give rise to the pharyngeal ring as an ectodermal thickening in the region of the pharynx. These proliferate internally and form eight ganglia (Pai, Ganin, Goethe, Strassen, 1892). According to Pai, this thickening can be easily confused with another in this region composed of mesoderm and ectoderm which will form the salivary gland. The formation of the longitudinal nerve ring follows immediately after the ectodermal thickening takes place, but the details of this process are not known. The nervous system of *Anguillula* is made up of 185 cells.

According to James (1892) and Pai, the excretory duct or lateral duct is derived from the ectoderm, in *Anguillula* the cells derived from the S_1 (AB) blastomere push into the body cavity and form this duct, with an opening at the side of the pharyngeal bulb. Hamann, Strassen and Corte state that it arises from mesodermal cells which appear in the pharyngeal bulb and extend in the form of a cylinder along the body wall. In more highly differentiated species, lateral canals starting at the posterior end run forward along the lateral lines at the two sides, to join in the oesophageal region, where the commonduct empties, into an excretory ampulla in the ventral mid-line. This canal system consists of four ducts, but there are many species differences and the details are not clear.

The S_2 (EMSt) blastomere gives rise to the mesoderm. This mesoderm grows in the form of bands which extend anteriorly and posteriorly between the endodermal and ectodermal cell walls. At a later stage the bands get loosened and the mesodermal cells disperse. The blastocoel or primary body cavity appears between the epidermis and the gut wall as the later take shape. The cells which lie scattered about in these regions form connective tissue and others in contact with the epidermis form the body musculature. Both types of tissues extend as fibres under the cuticle. The body wall musculature of *Anguillula* consists of 64-cells, and the connective tissue of 61. The space between the gut and the muscle layer disappears with the development of the reproductive organs.

In *P. equorum* and *Rhabditis* two cells called terminal cells appear immediately after the gastrulation. These lie anterior and posterior to the two primordial germ cells GI and GII. The cell which is at the anterior end gives rise to the somatic parts of the reproductive system and the posterior one gives rise to the reproductive cells. Pai believed that in *Anguillula* the S_5 (F) blastomere produces the reproductive system. The anterior cell derived from the S_5 (F) forms the somatic part of the ovary, while the posterior cell forms the oviduct, uterus and seminal vesicle in the female. In the male the anterior cell forms the vas deferens, seminal vesicle and ejaculatory duct and the posterior cell gives rise to the somatic tissues of the testis.

In the young worm the primordial germ cells GI and GII lie mid-ventrally in the centre or somewhat towards the posterior end of the body. These are larger than the other cells of body. At first these cells remain dormant. When the young worm starts moulting they become active and start dividing. The dividing cells envelop the GI and GII cells. The entire cell mass become elongated into a cylindrical structure. Eventually the anterior end bends backward. In *Rh. ikedai*, the gonopore opening is located at the center of the gonad rudiment, slightly posterior to the midpoint of the body. In other species the position may be different. The gonopore opens to the outside and leads inward to the vagina. In *Allantonema*, the ventral body wall gives out a small mesodermal protruberance which becomes connected with the uterus to form the vagina. According to Strabell (1888), the muscle cells of this region form the sphincter in *Heterodera*. In the males, after the first moult, the caudal end bends and the copulatory spicules and the genital papillae appear. In the anterior part of the cloaca is the rudiment of the rectum and posteriorly there is a pouch-shaped mass of the S_3 (C) line which will form the copulatory bursa. The spicules develop in the space between these two rudiments, with this the gonadal rudiment undergoes a striking development; a pair of chitinous spicules are formed, located in the dorsal side of the rectum. In *Rh. ikedai* chitinous ribs are also formed at the sides in the posterior end. The development of those structures elevates the epidermis and gives rise to the copulatory bursa. The caudal region is formed by successive moultings. After about four or five moults, the differences between males and females make their appearance. In many cases the male reproductive system is in the form of a tubular one, while that of the female is paired and complicated. With regard to the female reproductive system the nematode species can be divided into four types.

Type A-I: In these the gonopore is in the centre, the ovary extends anteriorly and posteriorly. The free-living nematodes *Diplogaster*, *Chromadona* belong to this category.

Type A-II: Gonopore at the centre but the anterior and posterior ends of the ovaries are more elongated and turned back toward the centre. The genera, *Actinolaimus*, *Rh. ikedai* belong to this type.

Type B: Only a pair of parallel ovaries as in the case of *Ascaris lumbricoides* and *Parascaris equorum*.

Type C: A single ovary with the gonopore at one end e.g. *Morhystera*, *Cephalobus*, *Rh. Dujardin*. Out of these four types A-II and B are common.

Larva: In the oviparous species like *Ascaris* the embryo develops outside the maternal body. In the viviparous and ovoviviparous individuals, the growth takes place inside the uterus. In the free-living species the number of eggs laid at a time are usually smaller whereas in parasitic forms they are more. In primitive forms like *Rhabditis*, *Enterobius*, *Strongylus* the number of eggs are particularly smaller. Growth generally takes place by direct development and except for the specialization of parasitic adaptation, there is no metamorphosis. During the growth, the embryo undergoes two or three changes as the result of hatching and moulting. The young ones of oviparous species undergo marked growth after hatching. The reproductive organs reach complete development in a comparatively brief time.

Studies of the hatching in Nemathelminthes have been made by Stewart (1921), Asada (1923), Ida (1930), MacRao (1935), Yoshida (1939), O'Connor (1951) and Takehashi (1953), but the mechanism is not yet thoroughly known. In the Ascarids the hatching occurs at the time of the first moult. The young of horse and pig ascaris will hatch in Ringer's solution and the egg membrane also dissolves in the hosts intestinal fluid, setting the young worm free. If this larva attained sufficient growth it will undergo a second moult *in vitro*. In addition to the state of larva, external factors such as the intestinal fluid, temperature, pH and the mechanical action of the digestive tract play a decisive role in the control of hatching.

The larva moults four times before it become an adult, some free-living species like *Actinolaimus* moults five times. The life time of a worm which moults four times can be divided into five stages; the stages to the fourth moulting are then called larval and the fifth is the adult stage. There are few changes in the morphology during the larval stages, since practically all the organs except those of the reproductive system are already present in the first stage. The reproductive organs develop, the ecdysis proceeds rapidly, then it will take only few hours to complete, it is slow, then it may go for months. At the time of ecdysis the larva becomes quiescent and rigid. Spaces appear below the cuticle, first in the head and then in the tail regions. The cuticle then becomes separated and finally torn by the movements of the larva. In some forms all the cuticle is shed at once, in others bit by bit and some species shed even the lining of the mouth and oesophagus. In the males of *Actinolaimus* the posterior end is renewed at the third moult. The larvae of some species form cysts under unfavourable environmental conditions, pass through a dormant stage as encysted or ensheathed larvae, midtiny when conditions become favourable. *Heterodera* is one form which passes through a long dormant period. In the species which undergo successive moults, the total length increases by about three times within a few days after the third moult. Among the parasitic species, ecdysis bears a special significance with respect to infection. The larva gives up its free-living habit at the time of the second ecdysis and waits for a chance to penetrate a host. If it does not find a host, it may fail to undergo the second moult and become ensheathed. In *Ascaris* the larva remains within the egg membrane for a chance to infect the host. In forms which parasitize a single host, the second and the succeeding molts take place within the host while in the forms where there is an intermediate host, the second stage larva encysts within its tissues and molts, waiting as a third stage larva for the chance to parasitize its final host. In other forms the larva does not encyst, but finds and penetrates its definitive host by its own exertions.

Nematodes are usually dioecious but some members are also hermaphroditic. The dioecious forms carry reproduction by copulation whereas the hermaphroditic ones by self-fertilization. Some forms reproduce parthenogenetically.

In the genera *Rhabditis* and *Strongyloides*, there are cases in which the parasitic generation is hermaphroditic and produces fertilized egg by self-fertilization. These develop into the Rhabditis-type larvae of the free-living generation which becomes dioecious adults. The off-spring of these worms become Filaria type larvae and enter upon a parasitic habit, completing the alternation of generations. The hermaphroditic individual in most such cases has undergone the change from a female, but sometimes males may transform into hermaphrodites. In *Rhabdias buforis* both females and hermaphrodites have two sex chromosome (XX) while the males have one X. When spermatozoa are formed by the parasitic hermaphrodite individuals, the spermatogonia, which are really female cells, have XX-chromosomes. However, one X-chromosome and others with none are formed. So in the free-living generation both males and females are formed, in spite of the fact that they are produced by self-fertilization. During the course of spermatogenesis, in the free-living generation, the spermatozoa lacking an X chromosome degenerate, as a result all the offspring are XX females, which enter the lungs of frogs and become the hermaphrodites of the parasitic generation.

Additional evidence comes from some species in which males and hermaphrodites are found existing together. These hermaphrodites produce sperm during their juvenile stages and after reaching maturity, eggs which develop into hermaphrodites following self-fertilization. This results in a gradual reduction in number of males. In some species these males completely lose the capacity to copulate and disappear. In *Rhabditis* hermaphrodites forms spermatozoa in the part of the body between the sperm receptacle and the oviduct. In *Rh. sechellensis* the spermatozoa, which are formed in a limited region in the lower part of the oviduct, mature first while *Rh. gurneyi* forms eggs and sperm together at first, and alternately later. The *Rh. ikedai* hermaphrodite resembles *Rh. sechellensis* in an early stage, but afterwards shows similarities to *Rh. gurneyi*. This large hermaphrodite has a much convoluted oviduct and reproduces prolifically. On the other hand *Rh. elegans* and *Bhadynema rigidum* have hermaphrodite of the male type. For example in *Bhadynema rigidum*, there are both male and female larvae, but the male acquires an ovary in addition to its well-developed testis and grows to the adult stage, while the female larvae disappear. Since the spermatozoa mature first in hermaphrodites of this type, the supply may be inadequate to fertilize the later ripening eggs, which then develop parthenogenetically. In *Rh. sehneideri* moreover, reproduction takes place solely by parthenogenesis. In *Rh. aberrans* the sperm pro-nucleus within the eggs fails to unite with the egg pro-nucleus and finally disappears and the egg develops parthenogenetically. Among the nematodes in general, there tend to be fewer males than females and the sex varies with such factors as species and strain, place and time of collection and conditions of culture. The sex ratio of *Rh. ikedai* has been found to vary with the strain.

6. *Bryozoa*

All the Bryozoa form colonies, each member zooid of which is very small, consisting of a polypide having a tentacular crown and a sac-shaped cystid. The cystid is composed of an endocyst and its secretory product, the ectocyst. This group is divided into the Gymnolaemata and the Phylactolaemata.

I. Gymnolaemata

The lophophore is circular and the tentacles grow in a single ring. There is no epistome, and no fusion takes place among individual zooids. Most of these animals are marine forms, with the exception of the two fresh water genera *Paludicella* and *Victorolla*.

II. Phylactolaemata

The lophophore is horse-shoe shaped, with the tentacles borne peripherally. There is always an epistome covering the mouth. The individual zooids are all of the same shape, these fuse together and have a common body cavity. All are fresh water forms.

The methods of reproduction found among these forms are various: larvae are produced by sexual reproduction on the one hand and colonies are formed by asexual budding on the other. In some cases these species also proliferate asexually by means of dormant buds of a peculiar type.

Embryological studies of this group were begun in the latter half of the nineteenth century by Nitsche, Metschnikoff and others.

SEXUAL REPRODUCTION

Fertilization and Storage of Eggs

The individual zooid is usually hermaphrodite. The testes usually arise as botryoidal structures from the epidermal layer of the funiculus. The ovaries are formed from the oral side of the inner epithelium of the endocyst. Both spermatozoa and eggs are liberated into the body cavity, where fertilization takes place.

Generally self-fertilization takes place but in species where the gonads mature at different times, cross fertilization takes place. In such cases the spermatozoa are said to enter through the inter-tentacular organ. The inter-tentacular organ opens to the outside of the body or through small tubules at the tips of the tentacles.

The fertilized eggs are liberated to the outside or retained within the parent body where they begin to develop. In species with a well-developed ooecium, the fertilized eggs are transferred through the genital pore to the ooecium where they are stored for long periods (Fig. 6.1). In the group Cyclostomata, large numbers of embryos are sometimes found in the ooecium. This is due to *Polyembryogeny* i.e. after an embryo has reached the morula stage, it divides into several bits, each of which develops independently into a secondary embryo. A repetition of this process results in the formation of tertiary embryos. Because of this more than one hundred embryos in various developmental stages can sometimes be found within the ooecium (Fig. 6.2). In the Phylactolaemata, the fertilized egg is taken into a cystic ooecium located among the zooids; here it continues its development, and the embryo receiving nutrient substances through a

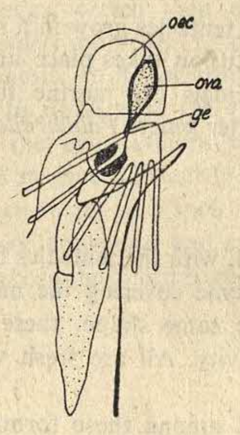


Fig. 6.1. Migration of ova in *Bugula*.
oec—ooecium.
ova—ovum.
ge—genital pore.

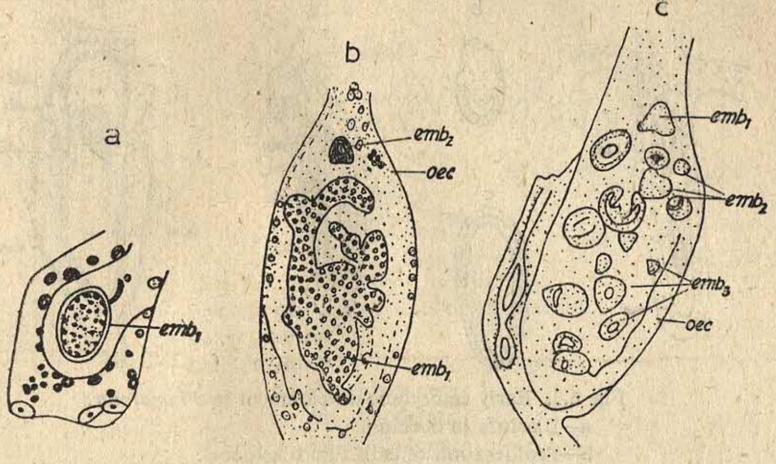


Fig. 6.2. Polyembryony in *Crisia*.
a—Ooecium containing morula-like primary embryo.
b—Ooecium with constricted primary embryo.
c—Ooecium with many secondary embryos.
oec—ooecium.
emb$_1$—primary embryo.
emb$_2$—secondary embryo.

placenta formed between the wall of the ooecium and the embryo (Fig. 6.3).

DEVELOPMENT OF THE EMBRYO

a) **Gymnolaemata**

The eggs of *Membranipora*, *Alcyonidium*, *Bugula* and *Paludicella* have small amounts of yolk. In these cases the cleavage furrow divides the whole egg almost equally. The 16-cell stage consists of four regular rows of four cells each. This plate of cells then divides horizontally so that the 32-cell stage is composed of two cell-plate layers. Soon these cell layers bulge outward, a cavity is formed between them and the embryo grows into a convex lens-shaped blastula. In the next stage granules appear in the four central blastomeres of the vegetal side, making them easily distinguishable from the other cells. These move inwards and fill the blastocoel. This constitutes the origin of the endoderm.

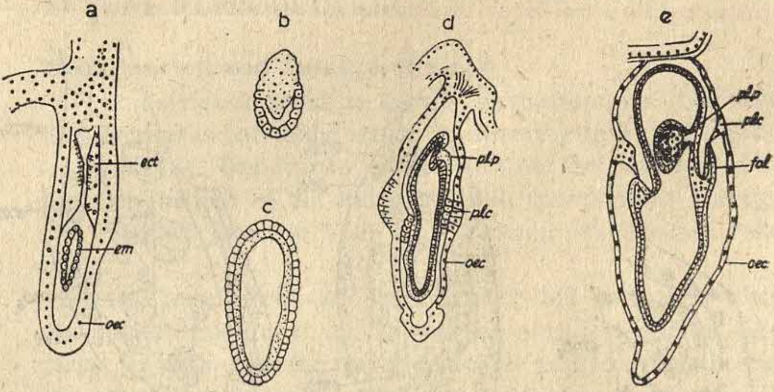


Fig. 6.3. Early embryonic development in *Plumatella*.
a—Blastula in ooecium.
b—Proliferation of cells into blastocoel.
c—Embryo with two layers of cells.
d—Formation of polyp rudiment and placenta.
e—Fully developed embryo.
ect—ectodermal cell.
em—blastula.
fol—ring fold.
oec—ooeceium.
ple—placenta.
plp—polyp rudiment.

The blastopore formed from this process is soon occluded by the surrounding cells. The surface where this is formed is called the oral surface.

The eggs of *Flustrella* have large amount of yolk and hence cleave unequally. As a result of this, at the eight-cell stage there are four large and four small blastomeres. A large blastocoel is formed at the 32-cell stage. The gastrula results from the migration of the large cells into the blastocoel (Fig. 6.4). This gastrulation is accomplished by a combination of invagination and epiboly.

In the genus *Alcyonidium*, as the development proceeds further, two characteristic cells appear at the two sides of the median plane. These are called as the mesoblasts which will later form the larval musculature. No such cells have been observed in *Membranipora*, but it is assumed that they appear in a similar way, since muscle tissue is formed in the larva. Prouho

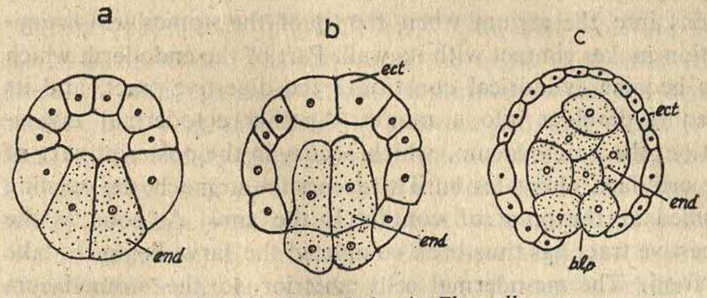


Fig. 6.4. Gastrulation in *Flustrella*.
a—Blastula with blastomeres of unequal size.
b—Early gastrula.
c—Late gastrula.
blp—blastopore.
ect—ectoderm.
end—endoderm.

(1892) suggested that these cells probably are derived from the micromeres at the oral face of the embryo or in other words from the ectoderm, since they lie in front of the mass of endoderm cells.

During this time the embryo is laterally compressed as a whole so that it takes a conical form, with the oral face as the base. The ectoderm at the tip of the core thickens markedly to form an apical organ and a large cavity develops in the oral face. This deepens and pushes the mass of endoderm cells towards the posterior as the first stage in the formation of the stomodaeum. At this stage the embryo still adheres to vitelline membrane by the margin of the oral surface and the apical organ, but a considerable degree of morphological change is about to take place. The apical organ acquires sensory hairs, the ectoderm around the oral face thickens into a mantle, the cells of which produce cilia to form a locomotory organ, when the mantle is folded, this portion is called the mantle fold. The whole of the oral face within the encircling mantle become depressed to form the atrium the deepest part of which becomes the stomodaeum. The ciliated cell-ring is called the corona.

The embryo at this stage breaks out of the vitelline membrane and enters the free-swimming stage. After this only the formation of the larval stomach begins. The endodermal cell mass lying in the blastocoel becomes hollow and then this cavity opens into the atrium when the tip of the stomodaeal invagination makes contact with its wall. Part of the endoderm which has become cylindrical constitutes the digestive tract, and its opening develops into a mouth. Another ectodermal invagination, the proctodaeum, which occurs in the posterior part of the oral face, elongates until it reaches the stomach; the opening formed at the point of contact is the anus. As soon as the digestive tract has thus been completed the larva begins to take in food. The mesodermal cells anterior to the stomodaeum proliferate and form a muscular string connecting the apical organ with the oral face. This is the rudiment of the larval dorsal muscles. Two thin, triangular shells secreted by the body wall envelop the larva; between them the apical organ can be seen. Among the Ctenostomata and Cheilostomata, a larva which has reached this stage of development is generally called cyphonautes (Fig. 6.5).

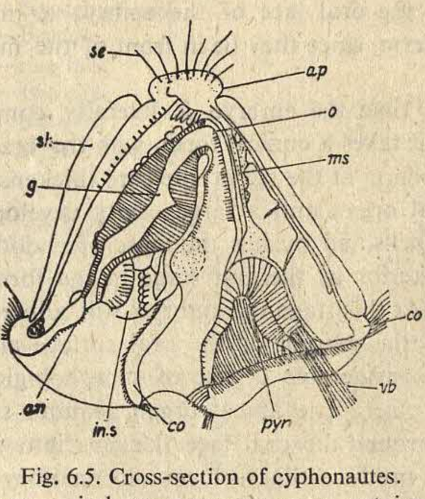


Fig. 6.5. Cross-section of cyphonautes.
an—anus; ap—apical organ; co—cornea; g—gut; ins—internal sac; ms—muscular system; o—mouth; pyr—pyriform organ; se—sensory hair; sh—shell; vb—vibratile plume.

Two kinds of organs are subsequently formed in the cyphonautes larva. One is the pyriform organ, and the other is the internal sac or sucker. The pyriform organ arises as an ectodermal invagination in front of the mouth. It consists of columnar cells, each of which has a glandular structure and carries on secretion. The surface of the organ is covered with fine cilia. Although these cells are continuous to the cell layers of the ciliary ring, their origin is different and their union with them is a secondary phenomenon. Anterior to this organ there is a group of long cilia bent like a hook, called the *vibratile plume*, the muscular string described above attaches at the base of this structure. Kupelwieser (1905) has suggested that the *vibratile plume* may be a kind of sensory organ with which the larva selects a place to attach when it enters the benthic stage.

The intestinal sac or sucker, is formed anterior to the anus. According to Kupelwieser, this results from an ectodermal thickening. Prouho held a different opinion, suggesting that it originates from an ectodermal invagination anterior to the anus. This structure is small at first, but develops gradually during the larval period into a large, sac-like organ with a thin upper wall and a glandular lower portion that secretes a mucous substance. These larval organs play important roles at the time of metamorphosis.

b) **Phylactolaemata**

In this group the ooecium is located between the individual zooids. The fertilized egg is taken into the ooecium and development takes place there. The investigations of Kraepelin (1892) show that in *Plumatella*, the number of blastomeres first increases by equal cleavage forming a blastula. Later the cells in the upper part of this embryo proliferate into its interior and fill the blastocoel, so that a coeloblastula results. These cells then proceed to shift to the periphery and leave the inner surface of the ectoderm, giving rise to an embryo with two cell layers and central cavity. The inner of these layers is mesodermal, the endoderm is believed to degenerate. The cavity which has appeared in the embryo therefore corresponds to the body cavity.

The embryo within the ooecium elongates. Its outer layer develops a band of enlarged cells and the cells of the ooecium lying opposite to them also protrude to meet them and form the characteristic placenta through which the embryo takes in nutrition (Fig. 6.3). A little later, a fold encircling the body wall of the embryo at the upper edge of the placenta form a ring fold. This corresponds to the mantle fold which appeared in the larva of the *Gymnolaemata.* Above the ring fold the outer layer of the embryo invaginates and gives rise to a *Polypide rudiment*. Before this primary polypide is completely formed, the outer layer invaginates again and forms a secondary polypide. At this stage periodic contractile movements begin near the polypide rudiments and propagate to the two ends of the body.

The polypide rudiments differentiate the various polypide organs by a process similar to that which will be described in connection with budding. The fully developed embryo is nearly oval and already bears two polypides. Entering the larval stage, it swims out through an opening left where zooids have died on the attachment region of the ooecium. In *Lophopodella carteri* the wall of the ooecium inverts as the larva escapes, protrudes outside of the body, and swells up like a baloon. It later shrinks gradually into a rod-like shape, and finally disintegrates.

Larva

Swimming larvae of various species can be caught with a plankton net. If colonies with well developed ooecia are collected, kept in the dark for a while and then suddenly exposed to light, the larvae all begin to swim simultaneously.

The cyphonautes larva of the Gymnolaemata differs markedly from the larva of the Phylactolaemata. However the comparison of various larvae shows that the gap between these two forms is filled by a graded series of developmental types and their associated morphological patterns.

The larva of *Memberanipora* is a typical cyphonautes (Fig. 6.5) and confirms in many respects to the fundamental structural formula peculiar to the bryozoan larva. The three germ layers are established early in its development, and the larval organs derived from the respective layers are formed as the larva begins its free-swimming life. The larva as a whole has a conical shape with two shells. At its top there is an apical organ and its lower margin consists of the ciliary ring. Since there is a complete digestive tract, the larva can take in the food. Muscles and nerves also develop and the larva has a pyriform organ and an internal sac. These structures are analogous in many respects to those of the trochophore and veliger. Viviparous larvae on the other hand, show many morphological variations which are correlated with the degree of viviparity.

The larva of *Alcyonidium* (Fig. 6.6) loses its shell, its apical organ expands and becomes disc-shaped and the mantle turns inward, forming a deep depression called the mantle hole. The oral face curves outward and the pyriform organ and sucker protrude. The digestive tract is extremely degenerate, consisting of only a stomodaeum and stomach.

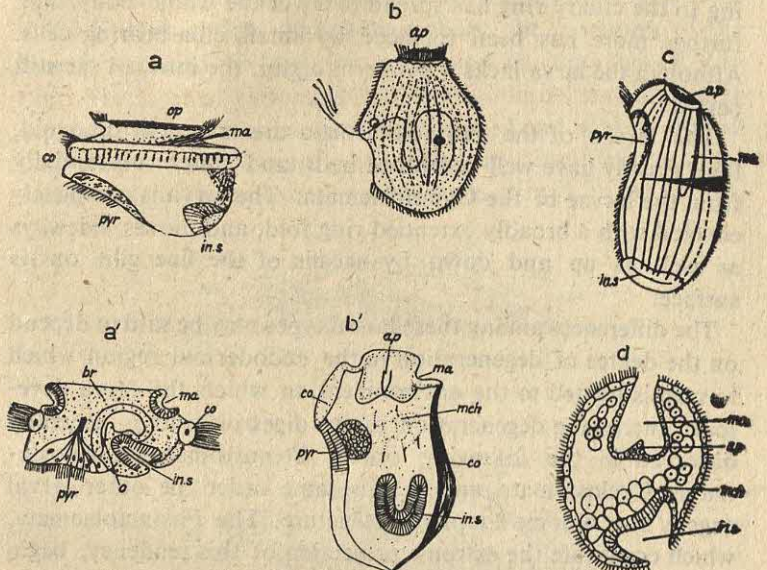


Fig. 6.6. Various types of larvae among Gymnolaemata.
a—Larva of *Alcyonidium*.
b—Larva of *Bugula*.
a′—Cross section of *Alcyonidium* larva.
b′—Cross section of *Bugula* larva.
c—Larva of *Serialaria*.
d—Larva of *Frondipora*.
ap—apical organ; ba—brain; co—cornea; ins—internal sac; ma—mantle cavity; mch—mesenchyme; o—mouth; pyr—pyriform organ.

A further degree of degeneration is observed in the barrel-shaped larva of *Bugula* (Fig. 6.6). This larva has a ciliated ring composed of a thick cellular layer covered with cilia, which encircles the body parallel to the main axis. The digestive tube has completely disappeared and the larval interior is filled with mesenchyme. The pyriform organ and internal sac persists. In *Serialaria* larva (Fig. 6.6) the portion bearing the cilliated ring becomes greatly extended longitudinally, and the larva as a whole acquires a very long and slender form.

In *Frondipora* larva (Fig. 6.6) the tendency toward degeneration is still more conspicuous. The body is ovoid, there is no trace of a digestive tract and the apical organ has lost its sensory cells and becomes a deep depression. The part corresponding to the ciliary ring has spread to cover the whole body, and further more has been replaced by small cilia-bearing cells. Although the larva lacks a pyriform organ, the internal sac still persists.

The larvae of the Phylactolaemata are spherical or ovoid, they already have well-developed buds, and differ substantially from the larvae of the Gymnolaemata. The larva is completely covered with a broadly extended ring fold, and moves sideways as well as up and down by means of the fine cilia on its surface.

The differences among these larval types may be said to depend on the degree of degeneration in the endodermal region which in turn is related to the environment in which the larva develops. Increasing degeneration of the digestive tract is generally observed in the following order: Ctenostomata—Cheilostomata—Cyclostomata, and in this same order the other larval organs also become simpler in structure. The Phylactolaemata, which constitute the extreme projection of this tendency, begin asexual reproduction earlier than do the other groups. When their larvae are being compared with the larvae of these groups, the discussion should be based on the cyphonautes, which has the most fundamental structure.

There are some Phylactolaemata in which no larva has yet been discovered. In such species it seems probable that the sexual mode of reproduction has in effect degenerated and been replaced by an asexual process.

METAMORPHOSIS

The length of the larval free-living period is correlated with the degree of development of the digestive tract. In the case of the cyphonautes larva where there is a well-developed digestive tract, there is a long free-living period, from a week to a month, during which the development of the incomplete organs proceeds. In larvae without a digestive tract, the free-living period is short. In the larvae of the Phylactolaemata the period is finished within two to three hours to half-a-day.

The cyphonautes which has reached the end of its free-swimming life begins to sink with its oral surface downward, and when it arrives at the bottom, it creeps about and selects a place to attach by moving the vibratile plume of its pyriform organ. Soon a strong contraction of the muscles causes the larval body to shrink. This brings about the extrusion of the internal sac, and the larva becomes fixed by it to the substratum. The layer of cells constituting this internal sac, or sucker expands and its outer margin turns upward and makes contact with the edge of the mantle, while the larva itself becomes flattened and acquires two covering shells (Fig. 6.7). After this, disintegration of the larval organs begins, the ciliary ring, digestive tract, pyriform organs, muscle fibres break up and their fragments accumulate in the lower part of the larva, where they are eventually consumed by phagocytes.

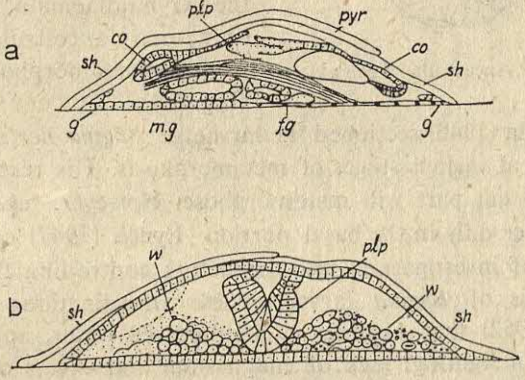


Fig. 6.7. Metamorphosis in *Membranipora*.
a—Flattened larva after attachment.
b—Larva in metamorphosis stage.
co—cornea; fg—foregut; mg—midgut; plp—polyp rudiment; pyr—pyriform organ; g—basal disc; sh—shell; w—degenerating tissue mass.

As a whole the larva acquires the form of a thin-walled sac, the layer of cells constituting the apical organ extends into the interior. The upper part of this structure becomes closed off, and it becomes the polypide rudiment. This, in the initial stages is composed of a single layer of ectodermal cells, but soon acquires a second layer. The origin of this second layer of cells, whether they arise from ectodermal cells or from mesodermal cells is not yet known.

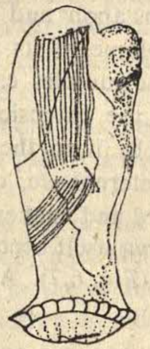


Fig. 6.8. Ancestrula of *Bugula*.

The polypide rudiment develops into the characteristic polypide form by a process similar to that found in the budding. The completed polypide, gradually everts its tentacular sheath, emerges out as a young zooid. The first zooid which makes its appearance is called the ancestrula. In the Gymnolaemata, usually only one ancestrula arises from the metamorphosed larva (Fig. 6.8).

Yamada (1940) sectioned the larvae of *Bugula neritina* horizontally, at various stages of metamorphosis. His results show that any cut part will metamorphose. However, regeneration takes place only in the basal portion. Lynch (1947) conducted a series of investigations into the factors controlling the metamorphosis of *Bugula* larvae. These investigations indicate (1949, 1952) that heating, hypertonic sea water, appropriate amount of lighting, lack of magnesium and excess of copper and of calcium, accelerate metamorphosis, whereas cold, hypotonicity, lack of light and excess of magnesium and potassium suppress it. He believes in general the accelerating and inhibiting agents are mutually antagonistic.

Since marine bryozoans are among the animals responsible for fouling ship bottoms, investigations into their larval metamorphosis have been made from the standpoint of fouling prevention. Mawatari (1951) has reported that mercuric compounds are the most effective agents for poisoning *Bugula neritinia*.

In the Phylactolaemata, the larva already contains two polypides, the rudiments of which are present even in the embryonic stage, indicating that the shift toward an asexual mode of reproduction is already recognizable during autogeny. After a short free-swimming life, the larva attaches to the bottom with its aboral pole. The ring fold slowly reverses, at the beginning exposing the first polypide and then the second. The ring fold gradually shrinks and becomes a lump of yellowish tissue. It is taken into the body cavity and is gradually absorbed as it circulates within the blood stream.

ASEXUAL REPRODUCTION

1) **Budding**

As the zooids grow, asexual reproduction brings about an increase in their number. The process of budding in these forms has been the subject of many papers. In the following description the budding of *Pectinatella gelatinosa* is given.

The bodywall of the zooid is composed of an outer layer and an inner lining epithelium or peritoneum. A bud first appears as a knob-like process, directed inwards, which forms on the oral side of the endocyst near its upper margin, and consists of a protuberance of some cells from the outer layer, surrounded by peritoneum. The outer layer of cells proliferate and soon a cavity is formed at their centre with the cells arranged around it. The bud thus grows into a cyst composed of an outer and an inner layer. Later the closed cyst develops an external opening and extends downward to become elongate and slender. Such a bud is still attached to the outer layer at one side, through the peritoneum. The portion of this membrane which lies between the outer layer and the bud gradually becomes thinner and at last breaks. The bud is then attached to the endocyst by its basal part and the portion adjacent to its external opening. A cord of tissue connecting the base of the bud with the endocyst, is the rudiment of the funiculus.

This long, slender cyst then proceeds to constrict in the middle and become separated into an inner chamber near the base and outer chamber adjacent to the opening. Later the inner chamber forms a digestive tube and the constriction between it and the outer chamber becomes a mouth. The outer chamber differentiates into a conical vestibulum, the wall of which will becomes the tentacular sheath. The cellular layer constituting the basal part of the outer chamber eventually thickens and bulges upward, and from each side of it a process arises toward the anal side. These extend in a semicircle and become the rudiments of the lophophore arms. Small, finger-like process, the rudiments of the tentacles appear on their upper circumferences. In the meantime, the inner chamber becomes still more deeply extended and differentiates into an oesophagus and stomach. An invagination from the stomach is directed upward, forming a rectum (Fig. 6.9). This makes contact with the wall of the tentacular sheath and then breaks through it to form the anus. The rudiment of the brain appears as a small invagination on the wall of the oesophagus, just below the mouth at the anal side. After this invagination is closed off, it separates from the oesophagus and forms the brain. From the two sides of this organ, horn-like process arise and extend into the lophophore arms to become the nerve trunks.

Immediately after the formation of the funiculus rudiment, the lophophore retractor muscles appear. A few cells of the peritoneum between the cystic bud and the outer layer become spindle-shaped and separate from the mother layer, forming muscle fibres which bind the middle part of the bud to the body wall. These, later develop into the retractor muscles. Similarly muscles located between the tentacular sheath and the body wall differentiate from the peritoneum.

In the meantime, the rudiment of the funiculus gradually elongates, a space arises in its interior and it develops into a tube. The growth of muscle fibres around this tube completes the formation of funiculus. In this case it may be said that the muscles and funiculus arise from the peritoneum of the endocyst.

As the bud takes the polypide form it emerges out by turning its tentacular sheath inside out. In an immature zooid which has just emerged, the tentacles are still immature, appearing as mere bumpy outgrowths at the tips of the arms. As the zooid feeds and continues to grow, the tentacles also develop extensively and the arms become beautiful horse-shoe shaped lophophores.

Each zooid bears pairs of buds in various developmental

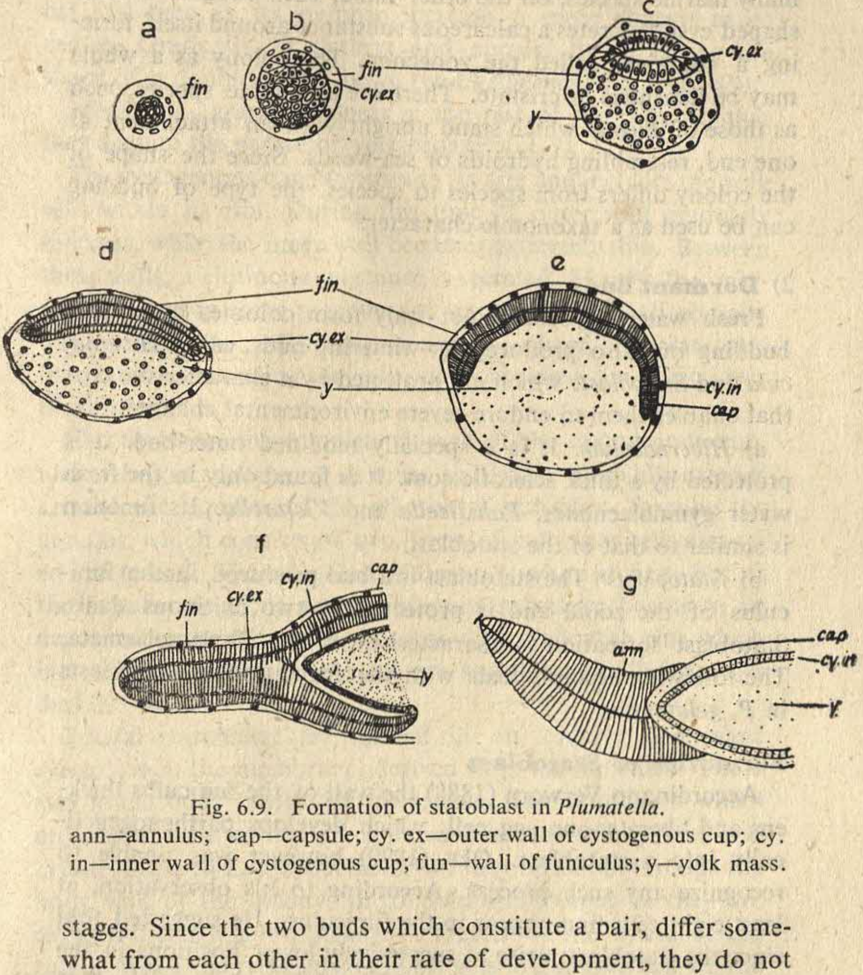


Fig. 6.9. Formation of statoblast in *Plumatella*.
ann—annulus; cap—capsule; cy. ex—outer wall of cystogenous cup; cy. in—inner wall of cystogenous cup; fun—wall of funiculus; y—yolk mass.

stages. Since the two buds which constitute a pair, differ somewhat from each other in their rate of development, they do not bud at the same time. Thus each fully grown zooid has two young zooids, one large and one small. As budding becomes active, the number of zooids increases gradually giving rise to a fan-shaped colony. Further increase in size leads to division of the colony and these divided colonies clump together into colonies. The colony of *P. gelatinosa* forms a gelatinous mass which may be as large as a human head. Among the freshwater forms such as *Plumatella*, there are many species which have a chitinous ectocyst and form branching vine-like colonies. In many marine species, on the other hand, each tubular or box-shaped cystid secretes a calcareous substance around itself, forming a container called the zooecium. The colony as a whole may be lichenous or cristate. There are also some species, such as those of *Bugula* which stand upright with an attachment at one end, resembling hydroids or sea-weeds. Since the shape of the colony differs from species to species, the type of budding can be used as a taxonomic character.

2) Dormant Buds

Fresh water bryozoans not only form colonies by asexual budding but also produce over-wintering buds, called *Hibernacula* and *Statoblasts* which are protected by a characteristic coat that enables them to endure severe environmental changes.

a) *Hibernaculum:* It is a specially modified outer-bud. It is protected by a thick sclerotic coat. It is found only in the fresh water gymnolaemates, *Paludicella* and *Victorella*. Its function is similar to that of the statoblast.

b) *Statoblasts:* The statoblast is a bud produced in the funiculus of the zooid and is protected by two chitinous shells. Statoblast formation is characteristic of the Phylactolaemata. The following account deals with the formation of statoblasts in *P. gelatinosa.*

Formation of Statoblast

According to Verworn (1888) the wall of the funiculus thickens and liberates one egg cell, which develops parthenogenetically into a statoblast. Oka (1890) however was unable to recognize any such process. According to his observation, at least eight cells first appear in the funiculus. He suggested that since the statoblasts arise at successively lower positions in the funiculus, they could not have originated from the inner wall of the stomach and must therefore stem from the body wall (ectodermal) connected with the lower end of the funiculus, or from the funicular wall (mesodermal) or from both.

These cells at first lie scattered free in the lumen of the funiculus; they gradually increase in number and come together into a morula-like formation (Fig. 6.9). This causes a local distension of the funiculus. Soon, certain special cells, which are clearly distinguishable from the others accumulate at one side of this mass. These groups of cells in the beginning are spherical and then as its number increase, a space appears at its centre. Gradually, it flattens and curves, finally taking the shape of a double-walled bowl, the *Cystogenous cup* (Fig. 6.9).

Meanwhile, granules appear in the rest of the cells, so that they acquire the nature of yolk cells (Fig. 6.9).

The cystogenous cup increases in its size and includes the yolk cells within its rim. During this time its outer wall gradually thickens, while the inner wall becomes extremely thin. Between these walls, a chitinous substance is secreted. Finally the margins of the cup come together and the thin inner wall completely encloses the yolk cells. In the completed statoblast, this thin layer surrounding the yolk mass is called the outer layer (Fig. 6.9).

Shortly before the complete closure of the cystogenous cup, a crest begins to form on its outer wall. This gradually extends around the equator of the ellipsoidal statoblast, forming the annulus, which consists of two layers of columnar cells arranged end to end. The statoblast rudiment is white at first, but as the annulus develops and a chitinous substance begins to be deposited in the cell walls, it takes on a yellowish tint which increases intensely until the mature statoblast finally becomes dark brown.

Several statoblasts are formed in an orderly downward succession in the membranes derived from the funiculus. These stay in the body cavity for some time. The statoblasts which are stored in the colony are released when the colony dies and disintegrates. In *Lophopodella carteri* they break out through the body wall of the colony. In *Storella* and *Plumatella* they are released through a vestibular pore located on the cystid of the zooid. When the released statoblast gets dried, each cell of the annulus becomes an air chamber, making the statoblast buoyant. The statoblast always has a capsule surrounding its contents and an annulus. The shape of the statoblast varies markedly according to the species. Even within the same species there may occur considerable variation. There is no annulus in the statoblasts of *Fredericella, Plumatella, Stephanella* and *Gelatinella*. The statoblast of *Lophopodella, Pectinella* and *Cristatella* have a well-developed annulus. It bears spines around its periphery and the spines sometimes have several hooks (Fig. 6.10).

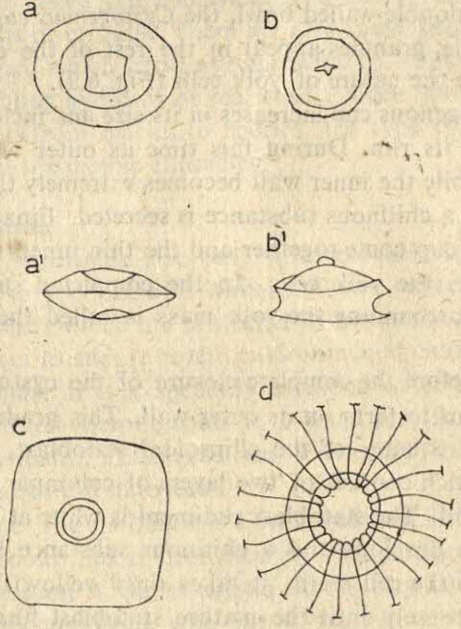


Fig. 6.10. Various types of statoblasts,
a—*Plumatella repens* dorsal view.
a′—*Pl. repens* lateral view.
b—*Gelatinella toanesis* dorsal view.
b′—*G. toanesis* lateral view.
c—*Pectinella gelatinosa*, dorsal view.
d—*Cristatella mucedo* dorsal view.

The statoblasts can withstand desiccation and low temperature. The power of resistance to desiccation differs according to species. In *L. carteri* almost all the statoblasts which have been desiccated and kept at room temperature for one or two years are able to germinate. After periods longer than this, the germination rate falls off abruptly. On the other hand, statoblasts can survive a considerable amount of cold. The members of these genera, over winter survive as statoblasts and the colonies die. However, statoblasts are formed extensively in summer also and so it is thought that their formation is not necessarily only for hibernation. The statoblasts have a period of dormancy; those formed in summer do not germinate until the following spring even if the conditions are favourable. These various considerations indicate that the freshwater bryozoans have adapted to their environment by making statoblast formation their principal method of reproduction. Since the statoblasts are always provided with a float or annulus, once they have been dried, they can be carried to great distances by water and wind or by attaching to water-fowl, and this must be effective in widening the distribution of the species.

Statoblast germination: In nature, the statoblasts of any given species germinate simultaneously in spring or early summer. Dried statoblasts readily germinate in the laboratory at any time if they are placed under favourable conditions.

The first sign of germination in *P. gelatinosa* is the separation of the two shell valves. Within the statoblast, a thin outer layer encloses a milky, granular, yolk mass. The cells lying along the equator of the ellipsoid of the outer layer begin to thicken and this thickening gradually propagates toward the polar sides. At one point in the equatorial plane, the cells become markedly columnar, form vacuoles, and take on the character of the outer layer in the zooid endocyst. The cells at the opposite end proliferate and extend into the yolk mass, producing a rod-like process in which they become regularly arranged around a space that opens in its centre to form a cyst. This is the polypide (Fig. 6.11).

In *Cristatella* and *Pectinatella* the outer layer on the dorsal side of the statoblast thickens to form a disc. On the surface of this a crescent-shaped hollow appears. When this hollow has developed into a deep invagination, the surface closes over it to form a cyst, which secondarily opens to the exterior. The early developmental processes thus appear to differ more or less in different species. In the meantime, a certain number of granule-containing cells have become arranged around the outside of the cystic rudiment, forming a peritoneum. The primordium of the polypide has thus acquired its two cellular layers.

The cystic primordium continues to extend more deeply into the mass of yolk, and then a median constriction divides it into inner and outer chambers. Each chamber differentiates as it does in the case of budding, to form a polypide that is, the

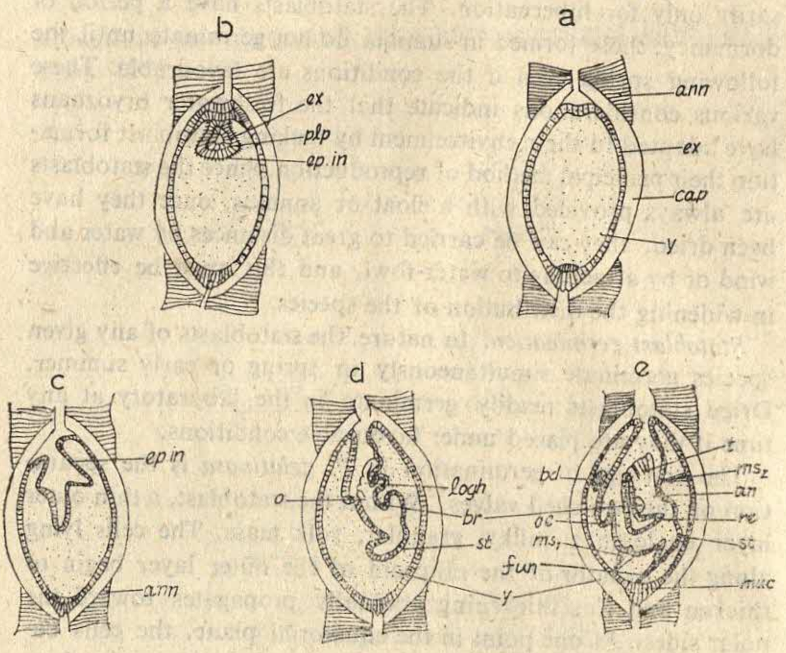


Fig. 6.11. Development of polyp in statoblast of *Plumatella*. an—anus; ann—annulus; bd—bud; br—brain; cap—capsule; ep. in.—inner lining epithelium; ex—external layer; fun—funiculus; loph—lophophore; ms—retractor muscle; ms_2—sheath muscle; muc—mucous pad; o—mouth; oe—oesophagus; plp—polyp rudiment; re—rectum; st—stomach; t—tentacle; y—yolk mass.

outer chamber becomes the vestibule, from the base of which the lophophore differentiates, while the inner chamber elongates to form the oesophagus and stomach. An invagination extending upward from the stomach develops into the rectum, completing the formation of the crooked digestive tract (Fig. 6.11). Only the mode of formation of the muscles and funiculus differs from that found in budding.

At the time when the anus is being differentiated the body cavity is still filled with a mass of granules. After that time, however, some granular cells between the tentacular sheath and the body wall, and others between the lower part of the oesophagus and the body wall, lose their granules and become spindle-shaped. It is these cells that develop into muscles. The former differentiate into the parieto-vaginal muscles, the latter into the retractor muscles. The muscles of the digestive tract and body wall differentiate from the peritoneum in a similar way. Simultaneously with the formation of the muscles, other granular cells become arranged in a cylindrical formation between the end of the stomach and the body wall. These cells are clearly distinguishable from the others by their marked stainability. These later lose their granules, and a space appears among them forming the primordium of the tubular funiculus. These granular cells are thus responsible for differentiating the muscles and the funiculus rudiment.

By the time polypide formation is completed, two cystic buds, a large and a small one, have already developed on the oral side of the body wall. The granular cells which have been filling the body cavity gradually disappear, although some of them acquire the nature of blood corpuscles. The body wall continues to grow increasing the size of the body cavity, and a mucous pad protrudes between the two shell valves and attaches the germinal body to some object (Fig. 6.12). This process in which the valves separate and the mucous pad protrudes through the space between them is called germination. On the following day the young polypide appears from the side of the statoblast opposite the mucous pad (Fig. 6.12). At the stage, the zooid

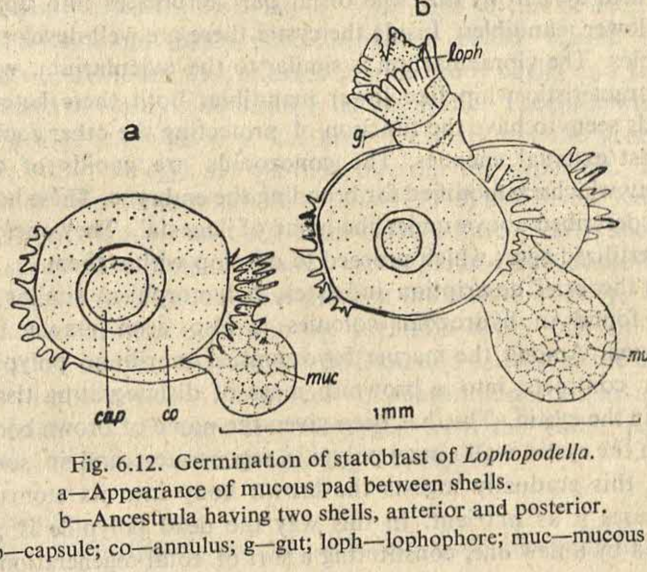


Fig. 6.12. Germination of statoblast of *Lophopodella*.
a—Appearance of mucous pad between shells.
b—Ancestrula having two shells, anterior and posterior.
cap—capsule; co—annulus; g—gut; loph—lophophore; muc—mucous pad.

resembles a brachipod, with anterior and posterior valves. The arms and body wall are still dotted with milky-white masses of yolk, these however, gradually disappear as growth continues. This ancestrula which has arisen asexually from the statoblast also forms a colony by a regular process of budding.

Polymorphism and Other Phenomena

The individual zooids constituting a colony typically proliferate at the expense of nutrients which they procure by the activity of their own tentacles. Among the marine bryozoans, some of the zooids differentiate along special lines. The atypical zooids thus formed are divided into kenozooids and heterozooids.

The kenozooid is a degenerated independent zooid which has lost its polypide. Inside such a zooid there still remain some epidermal cells and muscles. The chitinous or calcareous ectocysts of these zooids accumulate to form complicated structures which are useful for attaching the colony to various substrates.

The heterozooids include *avicularia*, *vibracularia* and *gonozooids*. The avicularium is a cystid, the bud of which develops in a special manner to resemble a forceps; this is seen among the Cheilostomata. The basic part of the cystid forms a stalk, and held upright by this, the distal part bifurcates into upper and lower mandibles. Inside the cystid there are well-developed muscles. The vibracularium is similar to the avicularium, with a characteristic whip like lower mandible. Both these heterozooids seem to have the function of protecting the other zooids against external enemies. The gonozooids are zooids of the colony which are modified for brooding the embryos. These have been described above under the name of 'ooecia'. They enclose the fertilized eggs, which proceed to develop within them.

As the brief description indicates, polymorphism similar to that found in hydrozoan colonies is also seen among the Bryozoa. Among the marine bryozoans, a moribund polypide often contracts into a brownish mass of disintegrating tissue within the cystid. This has been given the name of brown body. From the cystid wall a new polypide regenerates, and in some cases this gradually ingests the brown body into its stomach and uses it as nutrient. In this way the dead polypide is replaced by a new one, constituting a sort of 'total regeneration'.

7. *Phoronida*

J. Muller (1846) when first discovered the *actinotrocha*, which is the larval form of this group, he thought it represented an independent species and named it *Actinotrocha branchiata*. He regarded the ventral pouch in the abdomen as the reproductive organ. Later Schneider (1862) was of the opinion that this animal was a larval form belonging to the group Asteroidea. It was Kowalevsky (1867) who first showed it to be the larva of a phoronidean. He mistook the pre-oral lobe for the posterior portion of the body, and thought that the blastopore as such changed over to become the anus.

Fertilization

Most of the species of Phoronida are hermaphrodite excepting a few species like *Phoronopsis viridis*. The gonads develop on the coelomic epithelium near the ventral blood vessels. The mature gametes are first released into the body cavity and later projected to the outside through the nephridia. The place where fertilization takes place is not known exactly but the general belief is that the eggs are spawned through the nephridia. The work of Rattenbrug on *Phoronis viridis* (1953) and that of Kume (1953) on *Phoronis australis* from Misaki have clearly shown and supported a similar opinion once expressed by Kowalevsky (1867), that fertilization takes place in the coelomic cavity.

The fertilization occurs in the coelomic cavity late in the course of the first maturation division and as long as the eggs stay in the coelom, the process of maturation remains at a standstill. As the eggs are discharged from the body, polar bodies are formed and the eggs begin to cleave.

Cleavage

After the fertilized eggs are liberated from the body cavity through the nephridia, in many species they remain attached with mucus to the tentacular ring for a certain definite period. In the species *Phoronis ijimai* and *P. australis* the fertilized eggs develop here only into larvae with two pairs of tentacles. In the case of *P. viridis*, *P. mulleri* and *P. architecta* the eggs are spawned freely into the sea-water. In general, the eggs of the above three species are smaller than *P. ijimai* and *P. australis*.

Immediately after being released from the adult body, the fertilized egg forms the first polar body and then the second and in most cases the first polar body also divides at this time. During this period there can be seen encircling the polar bodies on the surface of the egg of *P. australis*, a ring of cilium-like processes which keep up a flickering movement.

The blastomeres are equal in size through the second cleavage, the first unequal division taking place at the third cleavage in such a way that the four cells of the vegetal half are somewhat larger than those of the animal half. Since the dividing nucleus lies in the animal hemisphere in *P. australis*, cleavage begins at the animal pole, as in the medusan *Spirocodon* and some others, and advances toward the vegetal pole while five protoplasmic processes arise at the animal pole side and bind the two blastomeres together.

According to the recent study of Rattenburg (1954) on *P. viridis*, this species clearly undergoes spiral cleavage (Fig. 7.1). Equal cleavage continues to the third division, resulting in eight blastomeres to approximately the same size but this cleavage takes place in a clockwise direction, so that the four cells of the animal side (First micromeres la, lb, lc, ld) are interposed between the four cells of the vegetal side (first macromeres lA, lB, lC, lD). Similar cleavages follow each other according to the general plan of spiral cleavage, alternately clockwise and counter-clockwise, and the divisions of all the blastomeres, taking place almost synchronously. The sixth cleavage, which results in the 64-cell stage, goes irregularly to some extent. Thus the fourth macromeres, which have just given off the fourth micromeres, are larger than the other blastomeres. At this stage the blastocoel is already extensive, and contains suspended fragments of cytoplasm.

The blastomeres derived from the first and second micromere group divide more rapidly than others from the seventh

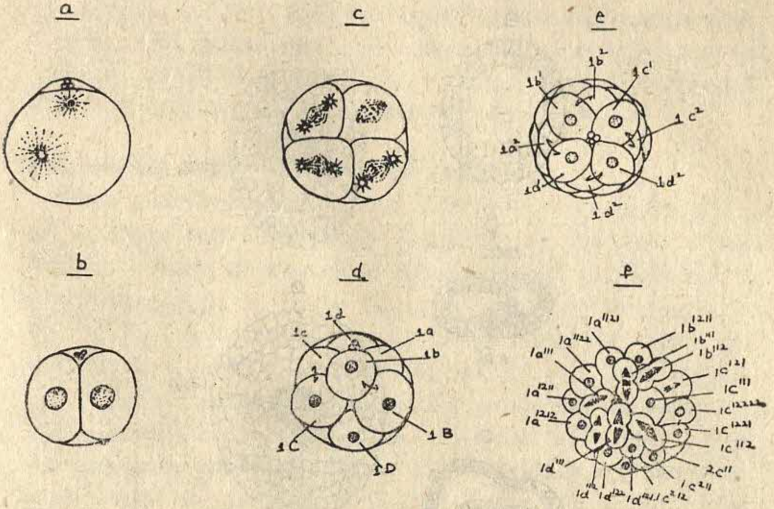


Fig. 7.1. Cleavage in *Phoronopsis*.
a—Fertilized egg.
b—2 cell stage.
c—4 cell stage.
d—8 cell stage.
e—16 cell stage.
f—Seventh cleavage.

cleavage onwards, and the macromeres divide at a correspondingly slower rate, so that it becomes difficult to follow cell lineage in these stages. The embryo undergoing eighth cleavage which is about 13 hours after fertilization bears a cilium on each of its cells. The blastomeres descended from the first and second micromere group form the dorsal side of the embryo and consequently become the very smallest of the blastomeres.

Gastrulation

The ventral surface of the embryo flattens and is called the ventral plate. This is derived from the relatively large fourth macromeres and relatively small fourth micromeres. Later, this region will differentiate into mesodermal and endodermal cells. Because of this, Ikeda (1901) called it the mesoentoblast. Soon, several cells at the anterior edge of the ventral plate sink into the blastocoel, these are mesoderm cells, most of which are derived from the descendants of the 'A' blastomere (Fig. 7.2).

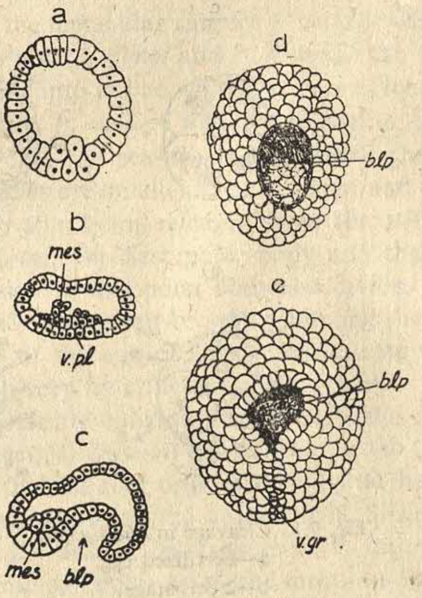


Fig. 7.2. Gastrulation in *Phoronopsis*.
blp—blastopore.
mes—mesodermal cell.
v.gr—ventral groove.
v.pl—ventral plate.

Next, the ventral plate begins to invaginate. The archenteron, formed by this invagination, the tip of which consists mainly of the large cells descended from the fourth macromeres, stretches diagonally toward the ventro-posterior part of the embryo. The blastopore when it is first formed is large and circular but gradually becomes ovoid. This may be due to the marked proliferation undergone by the ectodermal cells making up the dorsal side of the embryo (Fig. 7.2). The blastopore then gradually closes from the posterior side when it is completely closed, a small trace as the ventral groove can be seen. This ventral groove finally changes into a small triangular pore remaining open at its anterior tip, this later on changes into a lateral slit. When these changes of the blastopore are going on, the mesodermal cells are proliferating inside the embryo and at the same time become clearly independent from the endodermal cells. They spread throughout the blastocoel in the form of mesenchyme and part of this also encloses the archenteron wall. Among these mesodermal cells, there are none which can be specially identified as teloblasts, nor any which have changed over from ectoderm in the manner of ectomesoblasts.

Formation of the Actinotroch Larva

When gastrulation is completed, the embryo gradually takes on the actinotroch form which is peculiar to this group. This form is attained as a result of unequal growth and differentiation in various parts. In the first place, eight ectodermal cells (lq^{111} and lq^{112}) located at the top of the embryo just below the polar bodies, begin to elongate and produce long cilia, and an apical plate is formed with these cells as the chief components. The ectoderm between the apical plate and the anterior tip of the blastopore gradually spreads, until it extends over the blastopore like an umbrella and thus forms the pre-oral lobe characteristic of the larvae in this group (Fig. 7.3). As the pre-oral lobe elongates above the blastopore, the entrance to the latter gradually shifts forward, and a new larval mouth is formed at this point. The region between mouth and blastopore differentiates into the ectodermal oesophagus.

A ciliary band with long cilia is formed around the pre-oral lobe which corresponds to the prototroch of the trochophore. A similar ciliary band is also formed below the mouth and obliquely around the posterior part of the embryo. This is supposed to correspond to the metatroch. Tentacles appear along this metatroch. In the beginning only a single pair of tentacles appear on either side of the mid-ventral line and later two more pairs, one on the dorsal part of each side.

As growth proceeds, the embryo elongates downward, causing a stretching of the archenteron. At the blind tip of the lower part of this archenteron a cell-cord made up of several cells is formed. This cord transforms into a tubular intestine, and the archenteron itself will become the larval stomach (Fig. 7.3). The end of the intestine soon reaches the lower tip of the embryo, when an opening appears to become the anus.

Before the opening of anus appears, a small pit called as the nephridial pit appears in the ectoderm of the lowest part of the body, at the ventral side of the intestine. This pit deepens as

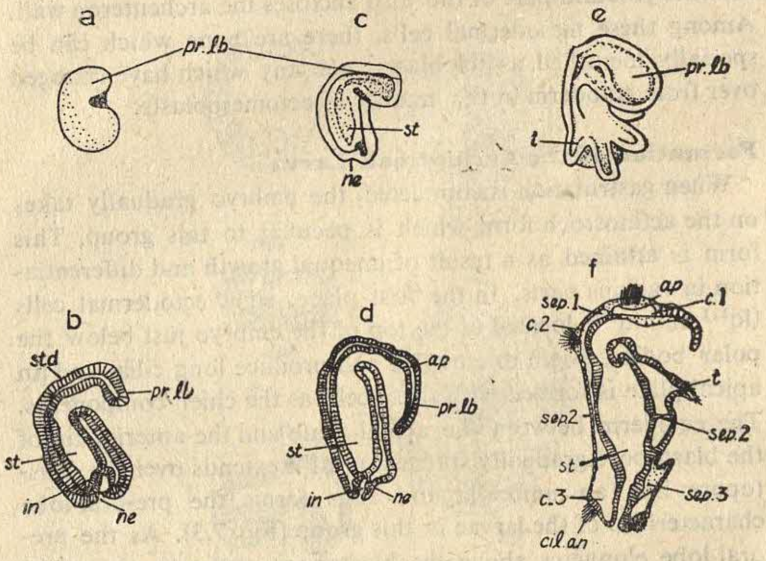


Fig. 7.3. Formation of Actinotroch larva.

a—Larva beginning to expand preoral lobe.
b—Longitudinal section of embryo at more advanced stage.
c—Larva at stage in which preoral lobe protudes and stomodaeum is formed.
d—Longitudinal section of embryo with 3 pairs of tentacles.
e—Embryo with 3 pairs of tentacles.
f—Longitudinal section of embryo at above stage 1.
ap—apical plate.
c1—pre-oral body cavity.
c2—collar body cavity.
c3—trunk body cavity.
cil-an—perianal ciliated belt.
in—intestine.
ne—nephridial pit.
pr.lb—preoral lobe.
sep. 1—preoral septum.
sep. 2—postoral septum.
sep. 3—ventral mesentery.
st—stomach.
std—stomodaeum.
t—larval tentacle.

the development proceeds and later elongates in a tubular form, its tip eventually bifurcating into right and left branches. When this happens the opening which had been single at first divides into two.

Ganglionic cells differentiate from the base of the apical plate and mesenchyme cells derived from the mesoderm proliferate, especially in the cavity included within the pre-oral lobe. When the larva remains in the parental tentacular ring, it begins its free-living stage in this condition. According to Hiraiwa (1925), the larva has a strong capacity for regeneration at this stage.

Free-living Stage

In the free-swimming larva the trunk continues to elongate and the number of tentacles gradually increase. The full number of tentacles varies from species to species ranging from 8 to 24 pairs. As the trunk elongates a new ciliary ring is formed around the anus. This perianal ciliated belt probably corresponds to the telotroch of the trochophore, plays an important role in the larval locomotion at this stage.

It is not known how many days are required before the larva can metamorphose. During the free-swimming period, the larva forms the various organs necessary for beginning its benthic existence. The formation of the larval tentacles is first completed and later the ectoderm at their bases begins to thicken and differentiates into the primordia of the adult tentacles. A more important structure is the ventral pouch, also called the metasome, which begins to be formed in a relatively early embryonic stage, as a thickening of the ventral ectoderm below the tentacular ring. This invaginates into the interior of the body to form a sac which grows around the digestive tract as development proceeds. Mesodermal cells become attached around the sac, these differentiate later into muscle tissue.

The nervous system of the larva is poorly developed and also that of the adult. No extensive differentiation of nervous tissue takes place, except for nerve fibres which grow out around the single ganglion formed below the apical plate. The branches of the excretory organ elongate gradually and at their coelomic ends, at the two sides of the stomach, flower-like groups of cells are formed. Muscular development is also poor, the retractor muscles at the sides of the oesophagus arise to the left and right of the ganglion and attach to the body wall near the tantacular ring. These muscles are used to move the pre-oral lobe.

Various complicated problems concerning the formation of the body cavity and the blood vascular system remain unsolved. The mesoderm cells proliferate in the blastocoel, some of them spreading throughout the cavity, in the form of mesenchyme cells, while others become epidermal and adhere to the body wall and the wall of the digestive tract, especially in the trunk region, where the body cavity becomes completely covered with this coelomic epithelium. Anterior and posterior septa are later formed across this coelomic cavity, dividing it into three regions, the pre-oral, collar and trunk coelom. The pre-oral coelom, which is the cavity of the pre-oral lobe, is filled with mesenchyme cells and the pre-oral septum, which divides it from the collar coelom is incomplete. The collar coelom is separated from the trunk coelom by the post-oral septum, a complete membrane formed posterior to the tentacular ring. The trunk coelom is thus an independent body cavity. Within this coelom, a ventral mesentery runs longitudinally along the ventral side of the intestine further dividing the ventral portion of the coelom into right and left cavities. The ventral pouch is formed along this mesentery.

Masterman (1898, 1900) who considered that the larva of *Phoronis* consisted of three body regions, pre-oral, collar and trunk, each of them including an independent body cavity postulated that the Phoronidae, like *Balanoglossus* belong to the Hemichordata. Ikeda (1901) however has suggested that it is difficult to regard the pre-oral and collar coelom as independent and maintained that the body cavities before and after metamorphosis have different origins. According to him, it is only the trunk coelom that remains as such after metamorphosis since the pre-oral coelom disappears with the resorption of the pre-oral lobe and the collar coelom shrinks and then changes into the ring vessel of the adult. He was of the opinion that although the collar coelom sends branches into the larval tentacles, the lophophore cavities of the adult are not formed by the retention of the collar coelom, but arise as new cavities from the bases of the tentacles.

In the pre-metamorphosis larva, the only blood vessel is the rudiment of the dorsal vessel, which is situated in the centre of the dorsal wall of the stomach, between the muscle tissue and the endoderm. However, some cells which can be identified as precursors of the blood corpuscles are already found scattered among the mesoderm eells of the early larva; these proliferate and eventually differentiate into two pairs of corpuscle masses at the sides of the stomach (Fig. 7.4).

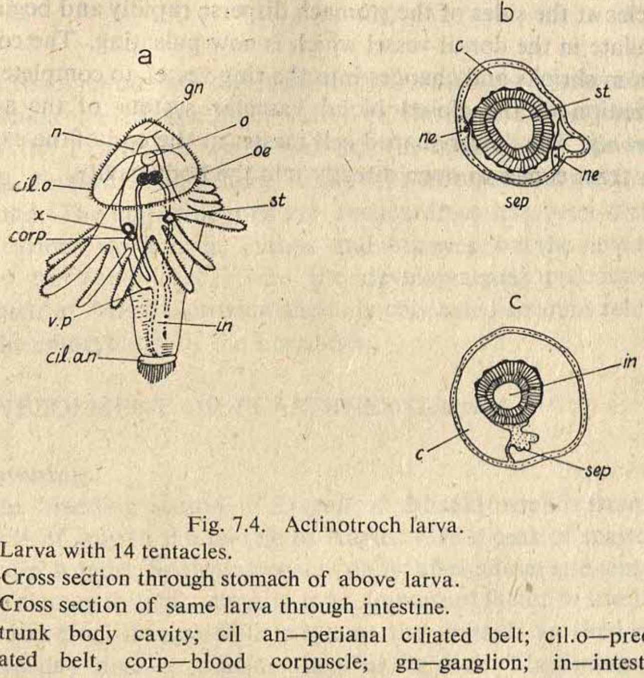


Fig. 7.4. Actinotroch larva.

a—Larva with 14 tentacles.

b—Cross section through stomach of above larva.

c—Cross section of same larva through intestine.

c—trunk body cavity; cil an—perianal ciliated belt; cil.o—preoral ciliated belt, corp—blood corpuscle; gn—ganglion; in—intestine; n—nerve fibre; ne—nephridial duct; o—mouth; oe—oesophagus, sep—ventral mesentery; st—stomach; up—ventral pouch; x—rosette cells of nephridial duct.

Metamorphosis

The metamorphosis of the actinotroch larva takes place rapidly, requiring according to Ikeda only 15-20 minutes.

The enlarged ventral pouch, folded within the trunk, is showing signs of evaginating; contractions of the trunk muscles cause it to evert so that the whole length protrudes to the outside. Since the tip of the pouch is connected to the digestive tract, all of the latter posterior to the stomach is drawn into the everted pouch. This causes the intestine to become U-shaped and the anus, which until now was located at the posterior end of the body, is shifted to a position near the tentacular ring. At this time the pre-oral lobe with the larval tentacles is absorbed into the larval stomach, and the perianal ciliated belt also proceeds to degenerate.

Within the body, in the meantime, the masses of blood corpuscles at the sides of the stomach disperse rapidly and begin to circulate in the dorsal vessel which is now pulsating. The collar coelom shrinks and changes into the ring vessel, to complete the formation of the closed blood vascular system of the adult *Phoronis*. The flower-shaped cell masses at the end of the excretory tract comes to open directly into the body cavity.

8. *Brachiopoda*

The Brachiopoda are divided into two orders: the Testicardines, in which the left and right valves of the shell are articulated, and the Ecardines, which lack such a connection. The lamp shell, *Terebratulina*, belongs to the first order, and *Lingula* to the second. The embryology of the Testicardines has been studied by various workers in Europe and America, but the investigations of Yatsu (1902) into the developmental processes of *Lingula* at Misaki provide the only connected account relating to the embryology of the Ecardines.

DEVELOPMENT OF ECARDINES (*Lingula*)

Spawning

The breeding season of *Lingula* at Misaki extends from the middle of July to the middle of August with a peak of spawning early in August. Spawning occurs daily, after sunset and sunrise; since change in light intensity is an important factor in stimulating spawning, it is possible to induce the animals to shed even in the day time by placing them in the dark. The females are induced to spawn by previous shedding of sperm by the males, but independent spawning by females is also observed on occasion.

The shedding of sperm and eggs provide a spectacular sight. If 10-15 animals are kept together in a glass container one male will usually begin to spawn, and keep it up for about an hour. The ejected spermatozoa rise in a white line for eight centimetres or more and if the animal is in a slanting position the white line may extend in a parabola to a distance of about ten centimetres. The gametes are expelled to the outside through one of the ciliated ducts which open along the anterior margin of the valves, after reaching this duct by way of the left or right nephridium; as a result the white line of spawned sperm can be seen to be formed by the union of two fine streams. This shedding of spermatozoa goes on for 20-30 minutes, as until practically the whole population of the container is shedding eggs or sperm. The eggs are shed in clumps, surrounded by a mucous substance; these clumps appear as a succession of dots when they are ejected from the ciliated duct. Each clump contains several pale yellow eggs.

Fertilization and Cleavage

The eggs are spherical, 0.09-0.10 mm in diameter, surrounded by a transparent vitelline membrane, and filled with pale yellow yolk so that they are opaque. There are radial streaks on the vitelline membrane, and the cortex of the egg contains from two to four layers of vacuoles. The sperm enters the vegetal side of the egg, and fertilization takes place at the telophase of the second meiotic division. The diploid number of chromosomes is 16. Since the polar bodies can be traced until at least the 32-cell-stage, it is possible, by using them as landmarks, to determine the egg axis and the order and direction of the cleavage. The mode of cleavage is extremely unusual, in general it resembles the cleavage of the bryozoan, *Bugula*. In its characteristics of biradial symmetry, is similar to that of the ctenophores. After fertilization, and before the first division begins, a marked constriction appears in the egg, this is not a true cleavage. The first cleavage plane includes the egg axis, and the second is perpendicular to this, also passing through animal and vegetal poles. The third cleavage plane is horizontal and perpendicular to the first and second cleavage planes. This divides the egg into eight blastomeres of about equal size. The fourth and fifth cleavages are peculiar. At the fourth cleavage two cleavage planes appear, parallel to the first. It produces two regular rows of four blastomeres each in both animal and vegetal hemispheres. The two planes of the fifth division are parallel to the second cleavage plane, forming in each hemisphere a regularly arranged layer consisting of four rows of four cells each, which gives the effect of a square floor laid with tatami. The first cleavage begins about an hour after fertilization at 27°C, and later cleavages take place at intervals of about 15 minutes.

After this stage, the cleavage processes have not been observed, but eventually the embryo expands into a spherical shape, and becomes a coeloblastula with a spacious blastocoel. The blastular wall is rather thick, on what is believed to be the vegetal side. An invagination appears in the centre of this region, and the embryo becomes a gastrula. Invagination takes place in the typical way, and the surface where it occurs corresponds to the anterior part of the future embryo. The cells making up the lateral walls of the invaginated archenteron proceed to divide and increase in size, to give rise to the mesoderm. They do not separate from the archenteron, but remain for sometime as a common cell mass with the endoderm. Yatsu (1902) called this the mesoentoblastic cell-mass. It is possible to differentiate between the two types of cells by differences in the staining properties of their nuclei. This meso-entoblastic cell-mass occludes the blastocoel and presses against the archenteron so that its lumen becomes indistinct and the blastopore is closed for a time.

Early Larva

It is after the gastrula stage has been completed, the embryo displays the special form peculiar to these species. First a circular fold of ectoderm rises around the embryo, this is equatorial and directed outward, and causes a shortening of the antero-posterior axis, so that the embryo is flattened into the shape of a disc (Fig. 8.1). This ectodermal fold is the rudiment of the mantle, while the mound shaped bulge which it surrounds on the anterior side of the embryo is the armridge, the rudiment of the arm region. Eventually a groove is formed on the anterior surface of this rudiment, which tends to divide it dorsoventrally. As development proceeds this groove extends to the posterior surface of the rudiment, completing the division of the mantle rudiment (Fig. 8.1). This change makes evident the difference between the dorsal and ventral surfaces of the embryo. The lower part of this divided mantle rudiment later changes to a flattened crescent shape as the result of extensive growth that takes place at the anterior edge of the embryo.

When the mantle rudiment begins to become separated into

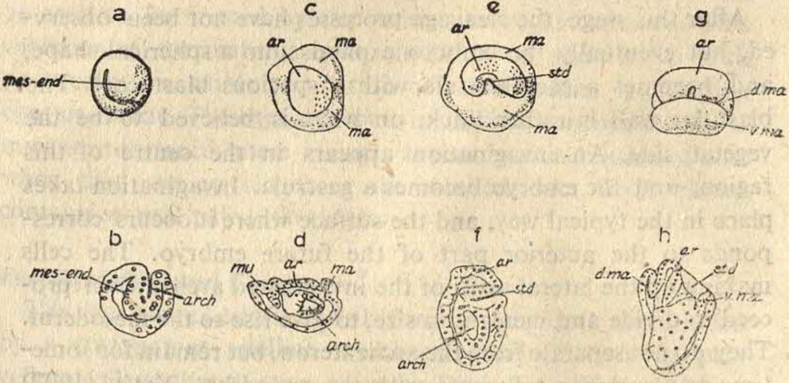


Fig. 8.1. Embryology of *Lingula*.

a—Late gastrula.
b—Longitudinal section of embryo in 'a'.
c—Frontal view.
d—Longitudinal section of embryo at stage of formation of mantle rudiment.
e—Frontal view.
f—Longitudinal section of embryo at the stage of stomodaeum.
g—Frontal view.
h—Longitudinal section of embryo undergoing marked growth of arm-ridge and mantle.

ar—armridge; arch—archenteron; d.ma—dorsal mantle: ma—mantle rudiment; mes-end—mesoendodermal cell mass; std—stomodaeum; vma—ventral mantle.

dorsal and ventral parts, a new invagination appears at the place where the original blastopore closed. This elongates in an antero-posterior direction and eventually its tip makes contact with the anterior end of the archenteron. The newly invaginated part is the stomodaeum which is of ectodermal origin, and will later form the larval oesophagus. The stomodaeal invagination causes the ventral part of the arm-ridge to assume a spread U-shape when viewed from the anterior side, but the whole armridge grows along with the growth of the mantle, causing marked changes in its form. As the arm-ridge increases in size, its front end expands into a mushroom shape and becomes flattened dorsoventrally. At the same time it shifts to a somewhat more dorsal position, coming to look as though it belonged to the dorsal mantle (Fig. 8.1). At this stage, the anterior edge of the ridge becomes densely covered with cilia. As these cilia begin to move vigorously the membrane which has been surrounding the embryo is broken, the rupture appearing first in the neighbourhood of the cilia.

About the time bumps appear on the anterior edge of the ridge, the protrusion which appears in the centre is the tentacle rudiment, and those at the left and right corners are the rudiments of the first pair of cirri (Fig. 8.2). In the interior, the

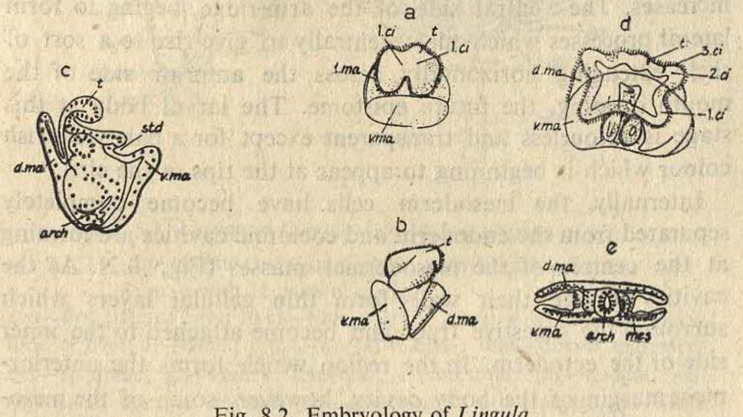


Fig. 8.2. Embryology of *Lingula*.

a—Appearance of tentacle and first pair of cirri.
b—Lateral view of same embryo.
c—Longitudinal section of same embryo.
d—Appearance of third pair of cirri.
e—Cross section of the embryo shown in 'd'.

1. ci.—first pair of cirri; 2. ci.—second pair of cirri; 3. ci.—third pair of cirri; arch—archenteron; dma—dorsal mantle; mes—mesoderm; std—stomodaeum; t—tentacle; v.ma—ventral mantle.

differentiation of muscle tissue is already taking place, so that the arm can be expanded and contracted. The secretion of chitin begins on the outside of the mantle. Internally, the lumen of the archenteron, which has established connection with the stomodaeum, begins to re-expand, and the differentiation of the archenteron wall produces a definite demarcation between stomach and oesophagus. At the two sides, the masses of mesoderm cells which separated from the wall of the archenteron are still lying without changes, in contact with the digestive tract. In this stage, the embryo sheds its membrane and takes up a free-swiming life.

During this stage, the number of cirri gradually increases, the new ones always appearing between the tentacle and the cirri which were formed first at the two sides. The number of cirri thus gradually increases from two pairs to three, four and so on. The central tentacle is a larval organ which begins to retrogress after the larva reaches a stage with 15 pairs of cirri, while the tentacle degenerates and the number of paired cirri increases. The ventral side of the arm-ridge begins to form lateral processes which meet centrally to give rise to a sort of shelf extending horizontally across the anterior side of the mouth opening, the future epistome. The larval body at this stage is colourless and transparent except for a pale yellowish colour which is beginning to appear at the tips of the cirri.

Internally, the mesoderm cells have become completely separated from the endoderm and coelomic cavities are forming at the centres of the mesodermal masses (Fig. 8.2). As the cavities enlarge, their walls form thin cellular layers which surround the digestive tract and become attached to the inner side of the ectoderm. In the region which forms the anteriormost margin of the body cavity, however, some of the mesodermal layer breaks up into mesenchyme. These cells invade the arm rudiment and part of them differentiate into the muscle tissue referred to above. When larvae are raised in the laboratory, their development stops at about this level, with not more than five to six pairs of cirri.

Later Larva

The rudiments of the major organs in general put in their appearance after the stage in which the larva has five to six pairs of cirri (hereafter referred to as the '5-6' pair stage). When it acquires ten pairs, a stalk (peduncle) projects from the posterior part of the mantle. At this stage the larva puts an end to its free-swimming life and becomes a bottom-dweller. The main changes which take place during this period can be summarised as follows:

The setae on the edges of the mantle, first appear at the seven-pair stage. These are secreted by the walls of ectodermal sacs formed as invaginations of the mantle (Fig. 8.3). The changes that take place in the digestive system are the formation of three liver lobes which arise as swellings of the stomach wall. The anterior and posterior dorsal lobes are formed from the dorsal side and the ventral lobe from the ventral side. The first of these to appear is the posterior dorsal, while the ventral lobe is formed last. The walls of the liver lobes are made up of polynucleate glandular cells and intracellular digestion is believed to be going on there. An intestinal tube connects with the

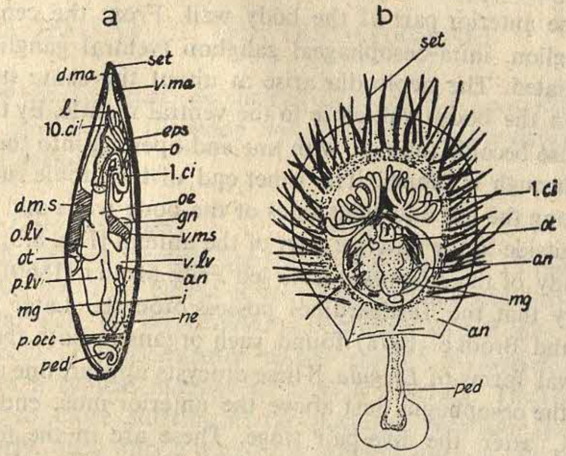


Fig. 8.3. Larvae of *Lingula* with 10 and 12 pairs of cirri.

a—Longitudinal section of larva with 10 pairs of cirri.

b—Larva with 12 pairs of cirri.

1 ci—first pair of cirri; 10 ci—10th pair of cirri; a.lv—anterior dorsal lobe of liver; an—anus; d.ma—dorsal mantle; dms—dorsal mesentery; eps—epistome; gn—ventral ganglion; i—intestine; mg—midgut; ne—nephrideal duct; o—mouth; oe—oesophagus; ot—otocyst; ped—stalk; p.lv—posterior dorsal lobe of liver; p.oce—posterior occlusor; set—setae; t—tentacle; v.lv—ventral lobe of liver; v.ma—ventral mantle; vms—ventral mesentery.

stomach; this opens to the outside by way of an anus in the 8-9 pair stage. The ectodermal stomodaeum gives rise to part of the oesophagus. The nuclear arrangement is different in this part from that portion of endodermal origin. The cilia of the two regions beat in opposite directions, the former beat toward the anterior while the latter beat toward the posterior.

A ganglion appears as early as the 5-6 pair stages as a thickening in the anterior part of the body wall. From the centre of this ganglion, infra-oesophageal ganglion (ventral ganglion) is differentiated. The nephridia arise at about the same stage as ridges on the body wall close to the ventral mantle. By the 7-9 stage these become tubular, with one end opening into the body cavity through a funnel. The other end of the tubule runs upward along the left and right sides of the body walls and opens to the outside in the anterior part of the animal (Fig. 8.3).

A study of the *Discinisca larva* led Fritz Muller (1860) to the discovery that the Testicardines possess otocysts. Later Morse (1881) and Brooke (1878) found such organs in both the adult and larval forms of *Lingula*. These otocysts appear, one at each side of the oesophagus just above the anterior-most end of the stomach, after the five-pair stage. These are in the form of spherical pouches 45-55μ in diameter in which about 40 minute otoliths are freely suspended. The cuboidal epithelium which makes up the walls of these vesicles arises by invagination of the ectoderm.

Muscle tissue of two types develops in the larva, the dorsal and ventral muscles belong to the larval type. The perpendicular muscles connect the dorsal and ventral valves of the shell and the oblique muscles between the valves persist into the adult stages. At the three-pair stage the ventral muscles develop at the two sides of oesophagus. At the 5-6 pair stage the dorsal muscles, anterior occlusors and oblique internus muscles are added, and in the 7-9 pair stage the posterior occlusors lateral muscles and oblique medius and externus are formed.

The stalk rudiment arises from the inner surface of the ventral mantle at its posterior end as a spherical bud which later grows into a twisted stem-like rod. The coelom penetrates into the interior of this structure to form the stalk cavity. Since the stalk is simply an extension of the body cavity its wall is composed of no more than an ectodermal epidermis and the coelomic epithelium, a small part of which differentiates into longitudinal muscle tissue. The rudiment protrudes to the outside in the ten-pair stage. The most striking change after this takes place in the ectodermal cells of the outer layer, particularly at the tip of the stalk, where they take the form of columnar epithelium and secrete an adhesive substance at the surface (Fig. 8.3).

DEVELOPMENT OF THE TESTICARDINES

Among the Testicardines such as *Cistella* and *Lacazella* have a brood chamber in the body wall, where the larvae develop for a certain period.

Early Development

As in *Lingula* the eggs are spherical (*Cistella*, 0.12 mm dia). It is known that they cleave equally and form a coeloblastula. The blastular wall in *Terebratulina* is composed of tall cells, and the blastocoel is consequently rather small. Invagination takes place in a typical manner to form a gastrula. The blastopore begins to close starting at the posterior side and proceeding anteriorly, until only a trace of it remains open at the front end, from which the closed part extends backward as a groove.

The coelomic pouches appear together with the mesoderm. In *Cistella*, two swellings of the archenteron wall project into the blastocoel about the time the blastopore is closing. These are separated off to become the coelomic pouches (Fig. 8.4). In the genus *Terebratulina*, a shelf-like process begins to extend from the anterior wall of the archenteron, dividing this structure into upper and lower parts. The smaller upper part will form the midgut, while the lower part spreads laterally to surround this from the sides and differentiate into the coelomic sac. When the embryo later undergoes considerable vertical flattening, the left and right sides of the lower structure become separated from each other as independent coelomic sacs. At the same time the upper structure is pushed against the ectoderm, and the blastopore is closed.

The anterior part of the embryo of *Cistella* (Fig. 8.4) is rather broader than the posterior. The transverse groove makes an appearance on its dorsal side and extends laterally (anterior mantle groove) at a position somewhat posterior to the place

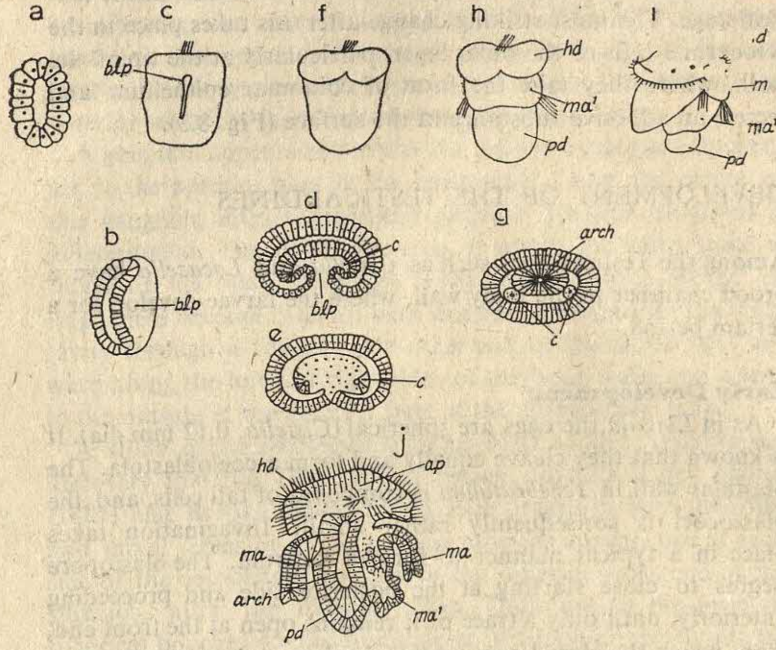


Fig. 8.4. Embryology of *Cistella*.

a—Blastula.
b—Gastrula.
c—Ventral view of gastrula.
d, e—Cross section of gastrula through region behind blastopore.
f—Embryo at stage in which formation of anterior mantle groove separates head from rest of body.
g—Cross section of the above embryo.
h—Embryo at stage in which formation of posterior mantle separates mantle from trunk.
i—Differentiation of mantle rudiment.
j—Longitudinal section of above embryo.

ap—apical plate; arch—archenteron; blp—blastopore; c—coelomic sac; hd—head region; ma—mantle; ma'—mantle region; pd—pedal region.

where the stomodaeal invagination will later occur (Fig. 8.4). This is followed by the formation of another groove behind the first one (posterior mantle groove), so that the embryo is divided into three parts consisting of a head portion, a central part enclosed in the mantle, and a posterior foot (Fig. 8.4). The mantle region elongates forming the mantle rudiment, and overgrowing the foot sooner, the dorsal and ventral sides of the mantle grow much more markedly than the sides, so that the mantle rudiment becomes divided into a dorsal and a ventral lobe. The setae which are formed on the borders of these lobes are larval organs, and will be shed at the time of metamorphosis.

The head portion eventually grows into a parasol-shaped structure with two eye-spots. Around the margin of the parasol there is a ring of particularly long cilia, while the ectoderm cells at its apex enlarge and form an apical plate. These structures and the way in which the cells at the base of the apical plate give rise to the super-oesophageal ganglionic mass are extremely reminiscent of the trochophore larva. The suboesophageal ganglionic mass differentiates from the ectoderm near the stomodaeal invagination in the basal part of the ventral mantle.

In both *Cistella* and *Terebratulina* the reopening of the stomodaeum occurs at the site where blastopore closure took place; internally an extension from the midgut unites with the stomodaeum, and this part becomes the oesophagus. No anus is formed in these species. Together with metamorphic changes of this kind, the coelom becomes greatly expanded, the cavities of the left and right sides meeting in the centre and uniting to give rise to a mesentery. Since the coelomic epithelium of the pedal region differentiates into muscle tissue, the coelom becomes indistinct here. When the foot later changes into a stalk, this muscle tissue forms the dorsal and ventral stalk muscles. On the ventral side of the embryo just below the ectoderm are formed the nephridia; these are differentiated from the coelomic epithelium as blind tubes which open to the outside at the base of the mantle.

Metamorphosis

In general, the free-swimming period is brief. According to Morse, the *Terebratulum* larva begins to attach to the bottom within 24 hours. The development of the arm organs is consequently delayed, so that they fail to appear in as early a stage as they do in *Lingula* and the only means of locomotion is provided by the ciliated band girdling the head region. As the tip of the foot begins to become attached to the bottom, the foot region transforms into a stalk, and the mantle, discarding its cilia, turns inside out so that it is directed upward to surround the head region, while the foot region is left exposed. At this time the setae which were present on the mantle are shed. Species which have setae as adults form them anew after this stage. Finally, the shell begins to be secreted from the outer lateral surface of the reversed mantle.

Eventually the head region begins to degenerate although a preoral process is formed from part of it. A horse shoe-shaped ridge appears on the inner surface of the dorsal mantle and its two ends extend around the mouth to become the rudiments of the arm organs. On this ridge the cirri will presently be formed bringing the shape closer to that of the adult arm.

Comparison with Ecardines

The formation of the preoral lobe and arm in the Testicardines is a rather different process from that of the Ecardines. There are still more fundamental differences to be found in the development of these two orders, to such an extent that some people even doubt that they are particularly closely related. The most important point of difference is concerned with the mode of formation of the mesoderm, *Lingula* is the only available representative of the Ecardines. The mesoderm arises as hickenings of the two side walls of the meso-entoblastic cell mass and the coelom appears later as schizocoel within these thickenings. In contrast to this, the coelom of the Testicardines is an exremely clear example of an enterocoel. Furthermore, the two orders have different modes of cleavage. In Ecardines it is somewhat special, showing radial symmetry, rather resembling bryozoan cleavage. In Testicardines it is said to show some traces of spiral cleavage.

The amount of research which has been devoted to the developmental problems of the Brachiopoda is still inadequate making it difficult to formulate definite judgements on the basis of present knowledge. More investigation is necessary to complete the account.

9. *Annelida*

The eggs of annelids are highly appropriate for embryological research, because of both the facility with which they are obtained and the fact that they can be fertilized artificially and their development can be followed up to the last stages.

The first embryological research on the annelids was descriptive. Nearly all the representatives of this important phylum were investigated. The most important investigations were done by Kowalewsky (1871), Hatschek (1878), Whitman (1878), Kieirenberg (1879), Salenksky (1882) and Goette (1882). In 1892 two more important papers were published by E.B. Wilson. The first one is 'The embryology of the earthworm' and the second 'The cell lineage of *Nereis*'. These two papers are considered as models for all ulterior research. The papers by Mead (1897), Nelson (1905) and Child (1900) closed the era of descriptive embryology. In the meantime experimental research developed.

ASEXUAL REPRODUCTION

The annelids generally have a great capacity for regeneration. In many cases from a cut fragment a new head or tail is regenerated. There are some annelids which solely depend upon this type of asexual reproduction. In forms like *Ctenodrilus*, one of the tube dwelling polychaetes and the oligochete, *Lumbricus* a wholly asexual process of monogamy takes place and there is said to be no indication of asexual reproductive process. In the syllids, both males and females separately undergo a process of schizogony by means of unequal fission or budding. After this the large numbers of male and female schizogonts carry on sexual reproduction.

SEXUAL REPRODUCTION

In the sexual reproduction, most annelids form a trochophore larval stage which metamorphoses into the typical vermiform adult. There are interspecies differences with respect to the characteristics of the trochophore, as well as the extent of metamorphosis. The developmental stages of Oligochaeta and Hirudinea take place in a protective cocoon. In these cases the larval period is secondarily reduced in relation with the change in environmental conditions caused by the development of cocoon. The larva grows into a vermiform adult within the cocoon. The development wherein free trochophore larval stage is present is called as indirect development and the development where the larval stage is passed within the cocoon and an adult hatches out of the cocoon is called as direct development.

The Archiannelida, Polychaeta and Echiuroidea are dioecious. Some hermaphroditic species are also known to exist. The gonads are formed in the wall of the coelomic cavity. The reproductive cells leave the gonads as soon as they ripen and fill the body cavity. They pass through the nephrideal ducts or special genital pores to the outside at the time of spawning. The gonadal tissue in many cases is formed only in the central segments. In the hermaphroditic species, half of the gonad-forming body region produces testes and the other half forms ovaries, the demarcation between the two is always distinct. In the dioecious species also sexual dimorphism does not usually appear. But with the advancing of the breeding season, the colour of the ripe gametes filling the coelom can be seen through the body wall, making it possible to distinguish between the sexes in a good many cases. In the sexual form of *Tylorrhynchus*, the body wall of the female is green, while that of the male is a pale pinkish-white. When *Arenicola cristata* arrives at the breeding season, the body colour of the female is purplish white and the male is white. Some species are known to show a strikingly conspicuous sexual dimorphism. The archiannelidan genus, *Dinophilus* and the echiroidean *Bonellia* are good examples of this. The males and females of these genera are so different that they were thought to belong to entirely different species. The females of these are 20 to 50 times larger than the so-called dwarf males. The males have undergone a regression in body organisation so that they are like parasitic forms in comparison with the females.

Among the Oligochaeta and Hirudinea, hermaphroditism is the rule. The gonads develop so that they overlap in the coelom and are confined to a small number of body segments. The male and female accessory reproductive organs lie in the adjoining segments. The reproductive openings of both sexes are on the ventral sides of special segments. Among the many oligochaet species a few cases have been found in which the sexes are separate.

REPRODUCTIVE SWARMING

In the Archiannelida, Polychaeta and Echiuroidea the ripe gametes are shed into the water and fertilization is external. In many pelagic polychaetes the annual reproductive season is limited to a very brief period and a conspicuous swarming phenomenon takes place in correlation with the lunar periodicity. During this time the sexually mature individuals (epitokes) appear simultaneously at the surface of the sea in tremendous numbers and swim about shedding their eggs and sperms. Such swarming as found among the polychaetes has been the object of attention because it occurs periodically every year with the precision of the lunar cycle. Remarkable breeding cycles dependent on the lunar cycle are frequently reported in species other than the annelidan, but there is probably no group in which such a large number of species show such beautiful lunar periodicity in their spawning as do the Polychaeta.

The first scientific report on the spawning and lunar periodicity was reported in the large sexual polychaete *Eunice viridis* commonly called the palolo worm. This worm is extremely long and slender (90×0.3 cm) with several segments bearing parapodia and setae and the body filled with mature gametes. This worm at the time of swarming abandons the head, retains the posterior three-fourths of the body and swims off as an epitoke. *Eunice viridis* lives in the coral reefs of Samoa, Fiji, and the island of the Gibert Achipelago in the South Pacific. Every year at about the last quarter moon of October and November i.e. on the eighth and ninth days after the full moon, during the twilight that precedes the dawn, a large number of these worms can be seen. These swarming palolo worms, once they have shed their gametes, promptly become the food of the fishes or sink to the bottom and die.

Other species which exhibit reproductive swarming equal to that in the Pacific are the Atlantic palolo and the Japanese palolo. The Atlantic palolo worm is the reproductive phase of the polychaete worm, *Eunice fucata* which occurs in great numbers around the Florida peninsula of the United States and near Puerto Rico in the West Indies. The Japanese polychaete, *Tylorrhynchus heterochaetus* also performs in a spectacular manner so that its sexual form has been given the separate name of 'Bachi' or Japanese palolo. The pronounced lunar periodicity of this swarming has been described by Izuka (1903), Oinuma (1926), Miyoshi (1939), Okada (1952), and Kagawa (1954). In this case the epitoke is formed by the head and the anterior segments. This worm lives in the sandy mud estuaries where the water is practically fresh. Every year myriads of them swarm after sunset for three days at the high water of the spring tides occurring during the tenth and eleventh lunar months. As they are carried by the outgoing tide towards the lower reaches of the river where the salt content is high, the eggs and sperms are shed from the posterior part of the body. Thus the bachi swarms twice in each lunar month, but the swarming which occurs at the dark of the moon is especially marked, while that at the full moon is clearly on. a smaller scale.

The Japanese brackish-water nereid, *Nereis japonica* swarms in the same way as the bachi during the twelfth and first months of the lunar calendar (Izuka 1908). In this case, the reproductive segments do not separate from the rest of the body, the whole animal, keeping the form of the sexually immature atoke, becomes filled with reproductive cells and swims to the surface to spawn. The gametes are shed through the nephridial ducts of each segment.

In addition to these examples, a considerable number of species among the lumbriconereids, nereids, perinereids, syllids and other groups are known to swarm accordingly to reports from Woods Hole (North America), Napoli (Italy) Concarnean (France), Madras (India) and the Malay Peninsula. The relationships between swarming habits and lunar periodicity differ somewhat with the species and localities. But in every case swarming is correlated with one phase or the other of the moon. The structural characteristics of the swarmers also show as many kinds as there are species. In some cases the complete atoke swims to the surface. In the bachi the entire post-body is cast off, and the palolo worm comes to the surface swimming without a head.

EGG LAYING AND BROODING HABITS

In almost all the sedentary and burrowing polychaetes fertilization takes place externally but swarming as is found among the Errantia is not seen in these groups. In many cases a large number of fertilized eggs adhere together to form egg-masses which are attached to the sea-bottom or some to objects like seaweed near the burrow. This type of spawning is seen in *Arenicola*, *Capitella* and *Sabella*. In the case of *Arenicola cristata*, the ripe gametes are shed from the body cavity through the six pairs of nephridia which serve as gonoducts, into the burrow where the animal lives. At the same time the body surface of the worm secretes a jelly like substance and the ripe gametes are caught in the jelly. The sperms may travel through this jelly to fertilize the nearest egg. A few hours later a large number of fertilized eggs make their appearance at the sandy bottom near the burrows of the females in the form of balloon-like egg-masses. The basal part of the egg-mass forms a slender stalk which extends into the sand as far as the burrow.

In *Arenicola claparedii*, the fertilized eggs are held together by the jelly into an egg-tube and go through their early development attached to the surface of the mother. In many species of *Spirorbis*, the early embryonic stages are passed in brood chamber formed by the operculum which is a modified tentacle. This brood chamber formation is not restricted only to the sedentaria but is found from time to time in Errantia also. In *Grubea limbata* and *Pionosyllis elegans*, the females carry the egg-masses attached to their dorsal surface. In *Polynoe cirrata* they are present on the dorsal cirri and in *Autolytus cornatus*, *Exogene germifera* and *Sphaerosyllis pirifera* on the ventral side. But none of these can be thought of having reached the full fledged brood cavity. As the brooding habit advances still further such a brood cavity comes to be developed inside the body. Such highly developed brood-chambers are rarely found in the Polychaeta. In the species *Syllis vivipara*, *Eunice sanguinea*, *Polydora ciliata* and *Pomatoceros triqueter*, the brood cavities are present, the males and females undergo pseudo-copulation.

In the tube-dwelling polychaetes also spawning habit is associated with the phases of the moon and tides. Regarding the spawning of *Arenicola cristata*, the spawning of the animals in any one habitat occurs at four-day intervals, at the same hour of the same day, and this periodicity coincides with one of the component curves which can be derived from the tidal periodicity curves. Moreover, the quantity of the spawning i.e. the number of eggs masses, fluctuates with the periodicity that follows the spring and neap tides, showing peaks when the moon is near the first and last quarter. In *Polyophthalmus pictus* and *Amphitrite ornata* the spawning is similarly correlated with a component tidal periodicity, showing a peak at a particular phase of the moon.

In the leeches and oligochetes the spawning habit is a little modified. These animals are hermaphrodite but generally cross-fertilization takes place. In the case of the common earthworm, *Pheretima communissima*, pseudo-copulation takes place by which sperms are exchanged and stored in the seminal receptacles. Pseudo-copulation has no direct effect on the fertilization. The ripe eggs are expelled through the female genital pore into mucus secreted by the clitellum. As soon as the eggs are shed the worm backs out of the encircling clitellum, simultaneously expelling into it from the seminal receptacle the sperms received during pseudo-copulation. The eggs deposited in the clitellum are externally fertilized with the sperms stored in the seminal receptacles. The mucus secreted by the clitellum forms the cocoon. Internally the cocoon is supplied with the nutritious albumen secreted by the epidermal glands of the clitellum. This slimy albumen not only supports the developing embryos but also supplies nourishment to the young ones. The number of eggs in a cocoon are generally few and the shape and size vary with the species.

The leeches have more complex reproductive organs. They are hermaphrodite with a penis and vagina. The fertilization is internal with the penis helping in the transfer of spermatophores to another individual. The fertilized eggs are placed in cocoons containing albuminous fluid. The size and shape of the cocoons change with the number of eggs that are present inside. In the fish leech, *Pesicola* the cocoon measures about 1 mm in length and contains only one egg. In the genus, *Hirudo*, the cocoon is barrel shaped, measures about 2 cm in length and contains about 20 eggs. The cocoon of *Clepsine* contains 200-300 eggs. Generally the cocoons of leeches are attached to aquatic plants or solid underwater objects or they lie scattered in the mud. But in the case of the genus *Clepsine* the cocoon is carried by the parent on its ventral side until hatching.

The organization of the egg: Lillie (1902, 1906, 1909) worked on the intimate structure of the egg of *Chaetopterus*. Raven et al. (1950) worked on the structure of the egg of *Sabellaria alveolata*. The first point to be noted is the activity of nucleolus during oogenesis. Martoja and Martoja-Pierson (1959) studied the nucleolus of the genera, *Pomatoceros* and *Hirudo* and found a large amount of SH-proteins in the nucleolus. During yolk synthesis these proteins are continuously passed into the cytoplasm. Srivastava (1952) who studied the nucleolus of *Lumbricus* found the transfer of granular nucleolar material through the nuclear membranes.

The second point which is of great importance is the origin, fate and function of 'yolk nucleus' or 'Blabiani body'. The work of Calkins (1895) on *Lumbricus* is of great importance. He noticed that the oogonia do not have a yolk nucleus. The yolk nucleus first appears as a heap of granules sticking to the nuclear membrane in the young oocytes. As the oocytes grow older this mass starts disintegrating and move away from the nucleus. This distintegrated mass would produce the yolk plates. When compact, the 'yolk nucleus' stains like chromatin and in the disintegrated state it stains like cytoplasm.

In unsegmented egg of *Nereis* and *Chaetopterus*, Spek (1930, 1934) observed that the animal cytoplasm shows an alkaline reaction, whereas the vegetal cytoplasm shows an acid one. According to the author this bipolar differentiation derives from a cataphoretic process at fertilization, as a result of which the positively and negatively charged colloidal particles are separated and accumulated from the poles. Later the ectoderm arises from the cells containing the alkaline cytoplasm and the entoderm from the cells which contain the acid cytoplasm.

Raven (1938) confirmed the bipolar distribution of the ooplasmic substances. He thought it to be due to a respective accumulation of the proteinic yolk in the vegetal region of the egg, and of hyaloplasm at the animal pole. Further studies of Raven revealed the presence of RNA in the unfertilized egg in the peripheral hyaline plasm, around the nuclear membrane and in the nucleolus. Glutathione is present in the cytoplasm. After the germinal vesicle has broken, glutathione spreads through the cytoplasm, an accumulation of it is observed around the spindle. Raven was unable to show that the substances segregated in the polar lobes were different from the rest of the cytoplasm. He concluded that the properties of these morphogenetic plasms must reside in some structural peculiarities of their proteins.

In the egg of *Diopatra* the RNA is distributed along an animal-vegetal gradient. Glycogen is scarce in *D. cuprea* but is found in large amount in *D. neapolitana*. Lipids are abundant. An important localization is that of the proteins and mitochondria at the animal pole. This localization is probably related to meiosis and initiation of the first cleavage. The young oocytes of *Autolytus* incorporate uridine-H^3 very actively. This incorporation is more at the places where large amount of RNA is present, particularly in the nucleolus. Full oocytes no longer synthesize RNA. Phenylalanine-H^3 is incorporated everywhere by the young oocytes, which means that proteins are synthesized everywhere. Fully grown oocytes do not synthesize proteins. The segmenting egg synthesizes them again and this synthesis is particularly active in the region of the spindle.

The polar plasms are particularly evident in the eggs of *Clepsine* and *Tubifex*. The Clepsine egg is released from the ovary in the first metaphasic stage. At this point no polar organization of the plasms can be observed. When the first polocyte is released the egg undergoes a contraction which redistributes the plasms. The contraction begins at the vegetal pole and gradually extends in the direction of the animal pole. After this contraction the egg resumes its spherical shape and orients itself with the animal pole uppermost. At this point two caps can be distinguished at the poles. The animal plasm has a circular configuration with the first polocyte at the centre, the vegetal plasm, on the other hand forms a compact cap. In *Tubifex* this distribution also follows a peristaltic contraction (at meiosis) which is so strong that it gives rise to the formation of lobes. Some lipids become segregated in these lobes. The significance of this fact is unknown. A very peculiar plasma segregation occurs in the eggs of *Chaetopterus*, *Sabellaria* and *Myzostoma*. Like some molluscan eggs these eggs form, before cleaving a cytoplasmic lobe at the vegetal pole. It is usually called as the yolk lobe or polar lobe. In Myzostoma (Fig. 9.1) the polar lobe is green, a red plasm is also present at the animal pole (Carazzi 1904).

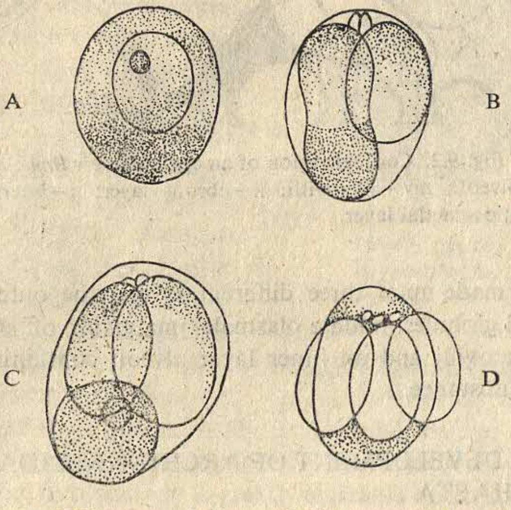


Fig. 9.1. Egg of Myzostoma before and after segmentation; the green plasm situated at the vegetal pole is segregated, at the 2-cell stage, in the polar lobe; at the 4-cell stage it is segregated in the D-cell.

The egg of *Nereis* has been investigated by Pasteels and by Fallon and Austu. According to these authors a differentiated cortex with many large alveoli is present. The alveoli contain a fibrous material, probably a precursor of the jelly coat. The cortex is limited by the plasma membrane from which many thick microvilli arise. These microvilli penetrate the perivitelline space and reach the chorion. Along the microvilli the jelly or its precursor which is found peripherally to the egg seems to be poured out (Fig. 9.2). The egg is surrounded by a thick chorion

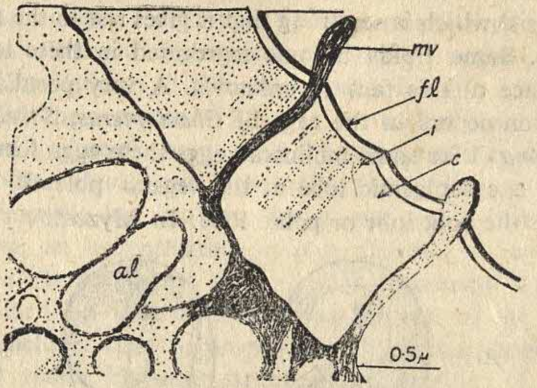


Fig. 9.2. Cortical region of an egg of *Nereis limbata*. al—alveoli; mv—microvilli; fl—fibrous layer; i—intermediate layer; c—caudal layer.

which is made up of three different layers, the outer one with spherical globules, middle plasmalemma giving off short cylindrical microvilli and an inner layer alveoli containing granular fibrous substance.

EARLY DEVELOPMENT OF ARCHIANNELIDA AND POLYCHAETA

Fertilization

The structure of the egg, as well as of its membranes change profoundly at fertilization. These changes were described accurately in the *Nereis* egg. Polychaete eggs usually have a homolecithal structure. Among the various species some have small eggs with clear cytoplasm and very little yolk. Others have large eggs with opaque cytoplasm. However, in most species the egg diameter lies between 100 and 200 μ; each species shows a beautiful colour tint of its own and finely granular homogeneous cytoplasm but many eggs also contain so-called oil globules in addition to the yolk granules. A well-defined membrane can always be recognized surrounding the egg, and in many cases a jelly layer is formed outside of this when the egg is shed. This jelly layer not only serves as a protective covering but it is also believed to have a close connection with the physiology of fertilization. In most species the egg is practically a perfect sphere, but it may occasionally be flattened in the direction of the egg axis or form a rather elongated oval structure. In all the cases, the egg nucleus, in the form of a germinal vesicle, lies more or less acentrically toward the animal pole.

The unfertilized egg of *Nereis* shows a distinct radial striation at the periphery due to a layer of alveoli (Fig. 9.3). At fertilization the alveolar layer disappears and a great amount of jelly is produced. The alveoli are situated inside the unfertilized egg and this view is supported by the observation of Novikoff (1939a). Costello (1949) also noticed that the alveoli can be displaced to the centrifugal pole by centrifugation.

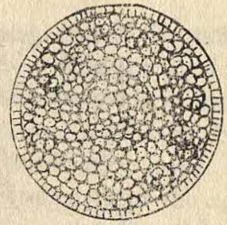


Fig. 9.3. Diagram of an unfertilized *Nereis* egg, the periphery is occupied by a sheet of vacuoles.

Another difference between the fertilized and unfertilized egg is the appearance of microvilli. In the unfertilized egg these microvilli are short and thick and in the fertilized egg they are long and sinuous. As a result of these microvilli the total surface of the fertilized egg is greatly increased and a connection between the exterior sea water and the interior of the egg is established. The unfertilized egg which remains in sea water for a while no longer exhibits the alveoli, all the perivitelline space being occupied by the remains (Fig. 9.4).

The fertilization does not change anything in the egg of *Sabellaria* except the microvilli are retracted. The spermatozoa are formed into spermatophores within the body cavity when these clumps of ripe sperms are shed into the sea water, they break up into active individual spermatozoa. The spermatozoa in all the species are flagellate, with a small head and a long tail, but individual characteristics can be recognized in the structure and size of the head region.

Among the Polychaeta fertilization usually takes place externally. The fertilization of *Nereis japonica* is described below as

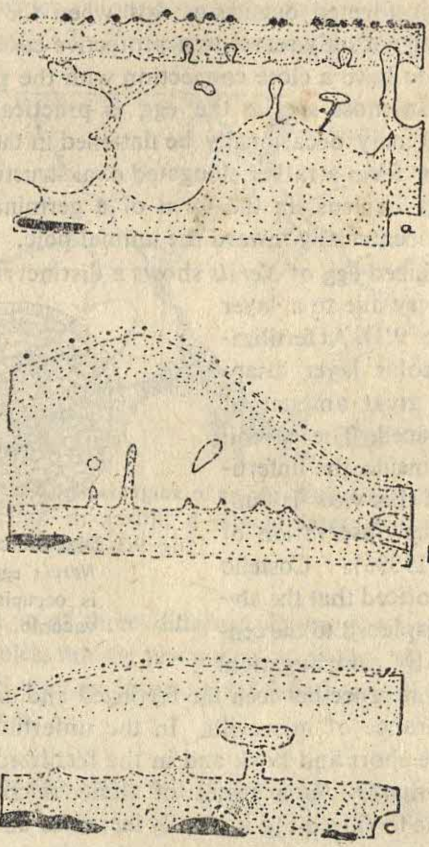


Fig. 9.4. Egg of *Sabellaria*.
a, b—Cortex and membranes in an unfertilized egg which was in contact with sea water for 30 sec and 45 sec.; c—The cortex and the membranes in a fertilized egg.

an example of the polychaetes. In the living condition the unfertilized egg of this species is rather flattened in the direction of the egg axis, the diameters averaging 160 × 170 μ. Under the egg membrane the cytoplasm is differentiated into a cortical layer which is 6-7 μ in thickness. At first glance this layer appears to be practically transparent, but on close examination reveals that it is densely filled with cortical granules which are in the form of short rods and particles about 1 μ in size. The endoplasm is pale green and contains opaque yolk granules. The germinal vesicle is surrounded by 30 or more oil droplets. The germinal vesicle itself lies eccentrically toward the animal pole and within it is the nucleolus. If the unfertilized egg is fixed with Champy's fixing mixture and stained according to Kull's method, the structure of the cortex and the endoplasm shows a different appearance depending upon the state of maturity of the egg. In the immature egg the yolk appears as small globules in the cytoplasm and the cytoplasm itself is granular. In the fertilized egg, cytoplasm is fixed in such a way that the yolk globules cannot be recognized.

If the eggs are inseminated in water, and if the salinity is between 12.28 and 17.7 per cent they will be fertilized readily and cortical reaction takes place. Insemination is immediately followed by changes such as formation of jelly layer, fertilization membrane and appearance of fertilization cone. As the material of the cortical granules is expelled from the egg, the cortical layer disappears, and finally the germinal vesicle breaks down. Simultaneously ooplasmic segregation takes place, an astral figure appears and very shortly afterward the polar bodies are extruded and the egg and sperm pronuclei unite.

When a spermatozoa makes a successful contact with an egg the contour of the egg undergoes a very slight shrinkage and the formation of the jelly layer begins. The thickness of this layer increases with time, so that it finally equals 1.5 times the egg diameter. In the case of *Nereis* no jelly layer is formed unless fertilization takes place, so it is possible to distinguish between a fertilized and unfertilized egg. At the point of sperm contact the fertilization cone appears, and with it the cytoplasmic cortex displays a fine structure of wavy lines while the cortical granules of the outermost layer begin to dissolve toward the egg membrane. At the same time a perivitelline space makes its appearance and the vitelline membrane is strengthened to form the fertilization membrane, while the remaining cortical granules show a new fine radial structure and continue to dissolve into the peri-vitelline space. All these cortical changes are completed within 20 minutes after insemination and the surface of the membrane regains its stretched spherical condition. If the cytoplasmic structure at this stage is examined in fixed material, it is seen that the membrane of germinal vesicle is still present, surrounded by a granular cytoplasm, and the small yolk globules again present a globular appearance. The thick, intricately formed cortex of the unfertilized egg disappears. These results indicate that the cortical granules of the unfertilized egg consist of precursor substances which contribute to the formation of the jelly layer and the strengthening of the fertilization membrane.

Thirty to 60 minutes after insemination the germinal vesicle breaks down and its membrane disappears. At about the same time ooplasmic segregation begins, the oil droplets and the yolk granules move to the vegetal hemisphere and the animal hemisphere becomes filled with the characteristic granular protoplasm. By about two hours after fertilization, a distinct astral figure is seen at about the centre of the egg. In this period the nuclear contents are eccentrically located at the animal pole, indicating the metaphase of the first polar body division. The aster disappears by about two-and-a-half to three hours after insemination, polar body formation is complete and the union of the egg and sperm pronuclei takes place.

Cleavage

A survey of the numerous papers which have been published about polychaete cleavage indicates that a common mode of cleavage is clearly recognizable. This is called 'annelidan cleavage'. It is total, unequal and spiral. The mode of cleavage in general, is always strongly influenced by the amount of yolk contained in the egg cell. When the amount of yolk is small, cleavage tends to be proportionally equal, but never loses its essentially spiral character.

A considerable amount of work has been done on the cell lineage of polycheates. The most important are the studies of Wilson (1892) on *Nereis limbata* and Woletereck's (1903) investigation of *Polygordius*. The following account of cell lineage is based on the development of *Nereis japonica* and *Nereis limbata* (Table 9.1).

The first cleavage furrow appears at the animal pole immediately after the polar bodies are given out. This first cleavage divides the egg into AB and CD blastomere. The AB blastomere is smaller than the CD blastomere. The second cleavage furrow appears 60 minutes after the first cleavage. It appears near the animal pole side and intersects the 1st cleavage furrow at right angles. This divides the AB blastomere into equal sized A and B blastomeres and the CD blastomere into a smaller C and a larger D blastomere. The blastomeres A, B and C are of about the same size. The blastomere A lies on the future left side of the embryo, B on the anterior side, C at the right side and D posteriorly. The third cleavage furrow appears 60 minutes after the second cleavage furrow and it is in a diagonally horizontal direction and separates off the equal sized micromeres 1a, 1b, 1c and 1d diagonally upward to form the eight-cell stage. The micromeres which contain no yolk are placed between the dorsal sides of the macromeres 1A, 1B, 1C and 1D forming the so-called first quartette of micromeres which surround the animal pole. The macromeres 1A, 1B, 1C are of the same size and 1D is fairly large. From this time the oil droplets present in the macromeres begin to be consumed. This causes the droplets to fuse together, so that they gradually become larger while their number decreases. In this third cleavage the micromeres are given off toward the right as seen from the animal pole. From this point it is possible to calculate back and formulate the hypothesis that the second cleavage is laeotropic and the first dexiotropic. As the cleavage progresses the successive divisions alternate their direction to form a spiral with the egg axis as its centre. In spiral cleavage it is a rule that the odd-numbered divisions are dexiotropic and the even numbered ones laeotropic.

At the fourth cleavage, the macromeres 1A, 1B, 1C and 1D divided obliquely toward the left and unequally, giving rise to the second micromere quartette, 2a, 2b, 2c and 2d. At almost the same time the micromeres of the first quartette divide equally and laeotropically, becoming eight micromeres ($1a^1$ and $1a^2$ . . . . . .1d and $1d^2$) as the embryo reaches the 16-cell stage when the second micromere quartette is formed, the D quadrant undergoes an atypical cleavage in producing the 2d blastomere. Although this 2d cell does not contain any yolk, it is equal in size to the macromeres. This 2d cell is destined to produce the ectodermal cells which later form the embryonic body wall of a wide area in the central posterior and ventral regions. Since this cell is separated off as the rudiment of the trunk ectoderm, it is known as the first somatoblast and is usually indicated by the symbol X or x. During the three following divisions the X divides unequally giving off 1x, 2x, 3x obliquely downward and then cleaves equally into X^l and X^r which lie symmetrically to left and right of the midline and constitute the teloblasts of the first somatoblast (Table 9.1).

At the fifth cleavage, the third micromere quartette is formed by dexiotropic divisions. From this stage the strict synchrony of cleavage begins to slacken, and discrepancies in the speed of division appear between one quadrant and another or between quartettes. In general, the micromeres of the first quartette and the first somatoblast cells divide more rapidly than the others. Also there is a tendency among the micromeres of the third quartette for the D and C quadrants to precede A and B in cleaving. As a result, the fifth cleavage can most accurately be described as first producing a 23-cell stage and then taking the embryo through a stage with 29 cells before it finally reaches the 32-cell stage.

Since the macromeres 3A, 3B and 3C are the mother cells which will give rise to the future endoderm, they are called the endomeres. 3D, on the other hand only becomes an endomere after giving off the micromere 4d at the succeeding division. This 4d cell, as the mother cell of the future mesoblast or second somatoblast is usually designated by M. The micromeres $2a^2$, $2b^2$ and $2c^2$ which are formed in the A, B and C quadrants at the fifth cleavage, are known as the left somatoblast, median somatoblast and right somatoblast respectively.

The sixth cleavage eventually gives rise to a 64-cell stage, but by this time the synchrony of cleavage is more than confused, so that the quickly dividing blastomeres are already dividing for the seventh time before the slow ones carry out their sixth division. As synchrony breaks down, the typical spiral cleavage configuration which has been visible until this time begins to be lost, the special cleavage modes of X and M are interposed, the arrangement of the blastomeres changes from quadrilateral to bilateral symmetry, the number of individual cells increases and the embryo arrives at the blastula stage.

The cleavages and the process of blastula formation are strongly influenced by the amount of yolk in the egg. The eggs of *Nereis* and *Arenicola* with lots of yolk form steroblastulae with practically no blastocoel. The eggs of *Polygordius* which have very little yolk, the spiral cleavage is regular and transparent coeloblastula with spacious blastocoel is formed. The information of the special cells, the trachoblasts, the teloblasts of the mesodermal bands and of the ventral plate in the stereoblastula of the *Nereis* was described by Wilson (1892). Woltereck (1903) has described the cell lineage of the coeloblastula of *Polygordius*. The following account gives the development of *Polygordius* as described by Woltereck.

The sixth cleavage gives rise to 64-cells. These form the fourth quartette and are formed by the laeotropic division from the macromeres. The first quartette micromeres divide for the third time, those of the second quartette for the second time and the third quartette for the first time and all towards the left. In the resulting embryo the animal hemisphere is made up of 32-cells, all derived from the first micromere quartette ($1q^{111}$ and $1q^{112}$, and $1a^{121}$ and $1q^{122}$, $1q^{211}$, $1q^{222}$). At the vegetal pole the macromeres (4A, 4B, 4C and 4D) and blastomeres of the fourth quartette (4a, 4b, 4c and 4d) are situated. The 16 blastomeres derived from the second quartette ($2a^{11}$ and $2q^{12}$, $2q^{21}$ and $2q^{22}$) and the eight blastomeres derived from the third quartette ($3q^{1}$ and $3q^{2}$) form a band around the equator.

As the embryo enters the seventh cleavage, theoretically each quartette should be proceeding to divide more or less together with the dexiotropic formation of the fifth set of micromeres. But in practice retardation in cleavage time tends to appear in one quartette or another and this might be due to the onset of the larval organ differentiation. From the animal pole a rosette of apical cells formed by the centrally located $1q^{111}$ group of first micromeres can be seen. The conspicuous cells of quartette $1q^{112}$ are interposed between the rosetts of apical cells. The conspicuous development of these four blastomeres is a characteristic feature of anneliden cleavage, together they are called the "anneliden cross". The blastomeres of $1q^{121}$ and $1q^{122}$ surround the anneliden cross. The blastomeres $1q^{211}$, $1q^{112}$, $1q^{221}$, and $1q^{222}$ which are in the equatorial band become the trochoblast. Among these blastomeres retardation of the cleavage first appears in the apical cells and the trochoblasts. The apical cells eventually form a bundle of cilia (apical tuft) which will develop into the apical organ believed to be the sensory organ of the trochophore larva. The trochoblasts give rise to the prototroch which is the larval locomotory organ. At this stage cilia start appearing on the blastomeres of the $1q^{222}$ and $1q^{121}$ groups. The movement of these cilia causes the embryo to rotate within its membrane and the increased rate of movement finally breaks the membrane. The time of hatching varies greatly from species to species. At this time the cilia simply occur isolated in the four quadrants but as development proceeds they become connected with each other and eventually form a complete prototroch which encircles the equatorial region of the embryo.

In the vegetal hemisphere, four radial zones and four interradial zones, one derived from each quartette can be seen. The micromere groups of the 64-cell stage derived from the second quartette ($2q^{11}$, $2q^{12}$, $2q^{21}$, $2q^{22}$) occupy the radial zones of the equatorial region. Of these the groups $2q^{11}$, $2q^{12}$ and $2q^{21}$ divide to form $2q^{111}$, $2q^{112}$, $2q^{121}$ and $2q^{122}$, $2q^{211}$ and $2q^{212}$; they then stop dividing for a while and make posterior lateral contact with the prototroch cells.

The members of the $2q^{22}$ group, which is to give rise to the first somatoblast, pursue rather different courses of cleavage depending upon the quadrant in which they lie. The A and C members of $2q^{22}$ carry out two successive divisions, the first horizontal and the second transverse, to form the blastomere groups $2q^{221a}$ and $2q^{221b}$ and $2q^{222a}$ and $2q^{22p}$. The B quadrant members ($2b^{22}$) divides horizontally forming the four blastomeres $2b^{221l}$ and $2b^{221r}$, $2b^{222r}$ and $2b^{222i}$. The quadrant blastomere $2d^{22}$ divides once into $2d^{221}$ and $2d^{223}$.

The cell groups $3q^1$ and $3q^2$ of the third micromere quartette lie in the interradial zones. The $3q^1$ cells of each quadrant cleave transversely to form $3q^{1a}$ and $3q^{1p}$ and thereafter stop dividing and become associated with ectoderm cells bordering the equatorial prototroch cells. The $3q^2$ blastomeres of the A and B quadrants undergo a transverse and then a horizontal cleavage giving rise to $3q^{2a1}$, and $3q^{2a2}$, $3q^{2p1}$ and $3q^{2p2}$. The quartettes $3q^{2a1}$ and $3q^{2a2}$ and $3q^{2pp}$ are produced by the transverse and horizontal divisions of the C and D quadrants. The quartettes derived from $3q^2$ are interposed between those arising from $2q^2$ to form an area of ectoderm surrounding the endoderm cells.

The radial zones are occupied by the blastomeres of the fourth quartette. When the fifth quartette is being formed, the A and C members of 4q undergo unequal transverse cleavage to form large $4q^a$ and small $4q^p$ blastomeres, while the cells of the B and D quadrants cleave equally and longitudinally into $4q^r$ and $4q^1$. As in *Nereis*, the 4d cell becomes the mother cells of the future mesodermal bands and is commonly called as the mesoblast or second somatoblast and is represented by the symbol M. The 4d soon divides equally and longitudinally and the resulting cells are called as the teloblasts of the mesodermal bands. The rest of the fourth and fifth quartette as well as the macromeres, are destined to be endoderm cells and all will take part in forming the wall of the archenteron. By the time the fifth quartette of micromeres is being produced in the vegetal hemisphere, the blastula as a whole can be said to have attained its full development.

In the 64-cell stage the blastula is flattened, the blastocoel is large. As the cleavage advances, the vegetal side becomes much more strongly compressed and the larval shape changes to that of a plate. The prototroch cells stop dividing and develop vesicles and lie at the equator of the embryo with their long cilia. The apical cells also produce long cilia. This extreme degree of flattening is a peculiarity of the *Polygordius* blastula, and does not generally occur to such an extent in the blastula of the other polychaets.

ARCHENTERON FORMATION

When once the blastula is fully grown, the cleavage activity for the most part shifts to the endodermal cell group and the ectodermal cells surrounding them. In this region, cell division continues actively forming a pouch shaped or solid cellular rudiment which protrudes from the vegetal pole in the blastocoel as the embryo makes progress toward a gastrular organization.

In the Archiannelida and Polychaeta the archenteron formation may take place either by invagination or epiboly. In the coeloblastula the cleavage of the endodermal cells takes place first and these move into the blastocoel to form the archenteron. In the stereoblastula, because of the large amount of yolk, the endodermal cells lag behind in their cleavage. The ectodermal cells divide rapidly, spread and envelop the endodermal cells, thus forming the archenteron. Most species produce the archenteron by a combination of invagination and epiboly. There is no clear line of distinction between the two processes, the mode of gastrulation being secondarily influenced by the amount of yolk.

In the *Polygordius* blastula where the fifth quartette of blastomeres have been formed, the endodermal cells lying in the plate of the flattened vegetal pole region continue to divide and invaginate into the blastocoel, giving rise to a sac-shaped archenteron. The surrounding ectodermal cells divide and extend over this region and close the blastopore. The first indication of the invagination appears in the cleavage of the macromeres after they have released the fifth micromere quartette. For the first time the cleavage of 5A, 5B, 5C and 5D does not show the spiral cleavage pattern but shows a bilaterally symmetrical relation to the chief embryonic axis. 5A and 5C divide unequally and transversely into $5A^a$ and $5A^p$, $5C^a$ and $5C^p$. 5B divides equally and longtudinally into $5B^l$ and $5B^r$. 5D alone divides in a peculiar fashion. First it divide into $5D^1$ and $5D^2$. $5D^1$ next forms $5D^{11}$ and $5D^{12}$. The nine cells formed by this cleavage show a tendency to invaginate into the blastocoel at the central position occupied by $5D^2$ and $5D^{12}$. The micromeres of the fourth and fifth quartettes also divide successively transversely or longitudinally and push against the endoderm cells from the periphery and gradually crowd them into the blastocoel. As the invagination proceeds, the margin of the blastopore becomes distinctly visible. At this stage the blastopore is in the form of an elongated opening. The cells $4b^r$ and $4b^l$ form its anteriormost margin and $4d^r$ and $4d^l$ form the posterior side. The left margin is formed by $5a^1$, $5a^2$, $4a^9$, $5d^{12}$ and $5d^{11}$, $5b^1$, $5b^2$ $4c^9$, $5c^{12}$ and $5c^{11}$ form the right margin.

As the invagination advances the two cells $4a^a$ and $4c^a$, which lie at the centres of the two sides of the blastopore draw together. At the same time the ectodermal cells grow from the two sides toward the midline so as to cover the blastopore. In this stage, its outline still has the form of the figure 8, but as the overgrowth of the ectodermal cells continues, it closes as though the sides were being stitched together, except for small openings left at its anterior and posterior ends. Even afterwards the anterior opening is not completely closed. The ectodermal cells which are adjacent to it invaginate and form the stomodaeum here. The blastomeres $2b,^{211}$, $2b^{212}$ and $2b^{222r}$ and $2b^{222l}$ form the anterior wall of the stomodaeum, the cells $3a^{2a1}$, $3a^{2p1}$, $2a^{221a}$ and $2a^{222a}$ form the left side wall, the right side wall is made up of $3b^{2a1}$, $3b^{2pl}$, $2c^{2219}$ and $2c^{2229}$ and the posterior wall by $3c^{2a1}$ $3c^{2p2}$, $3d^{2a1}$ and $3d^{2a2}$. The ectodermal blastomeres $2a^{221p}$, $2a^{222p}$, $2b^{2a2}$ $2b^{2a2}$ move into the blastocoel at the time of stomodaeum formation and become the larval mesenchyme.

The posterior opening of the blastopore is soon covered over by the ectoderm but reopens when $2d^{222}$ and $2d^{221}$ give rise to the proctodaeum. The teloblasts of the mesodermal bands M^l and M^r as well as $3c^{2pa}$ and $3c^{2pa}$ develop into the left protonephridium. The blastomeres $3d^{2pa}$ and $3d^{2pp}$ will form the right protonephridium. All these cells show signs of moving into the blastocoel. When the process of invagination is completed, the endodermal cells give indications of their forthcoming differentiation into stomach and intestine.

This description of archenteron formation in *Polygordius* is not applicable to all Polychaeta. In genera where there is no invagination, the archenteron is formed only by epiboly. Each genus shows certain secondary changes and these are correlated with the amount of yolk present in the egg. In the genus *Arenicola*, a wide open blastopore is present in the endodermal cell mass. The growth of ectodermal cells causes an elongation of the larva in the ventro-posterior direction. The blastopore is closed at a later stage by the overgrowth of lateral and posterior ectoderm.

As gastrulation continues and the vegetal pole region moves into the blastocoel, a new dorso-ventral embryonic axis is established. The digestive tract which produces a mouth and an anus elongates in the antero-posterior direction along the ventral side and according to the new axis, the animal pole side of the egg is converted into the anterodorsal region.

ARCHIANNELIDAN TROCHOPHORE

When gastrulation is in progress cilia start appearing on the surface of the gastrula. These cilia form the organs of locomotion. Shortly afterwards the gastrula becomes a free-swimming larva, begins to secure food for itself and grows. In many species, the apical cells of the gastrula gradually become thickened and form an apical organ equipped with bundle of long cilia. The prototrochal girdle develops from the prototrochal cells and encircles the equatorial region. The descendants of the first somatoblast develop into the ventral plate which comes to have the stomodaeum at its anterior end and the proctodaeum at its posterior end. The larval organization progresses in this manner until a characteristic *trochophore* is formed.

The young or prototrochophore usually shows the following characteristic features. It has an apical organ and a prototrochal girdle. It has a digestive tract with the mouth and anus already determined (Fig. 9.5). In some species the apical tuft secondarily shows a tendency to degenerate. The upper part of the larva between the prototrochal girdle and the apical organ is called the episphere or *head blastema*. The hyposphere, below the prototrochal girdle arises as the result of the complicated

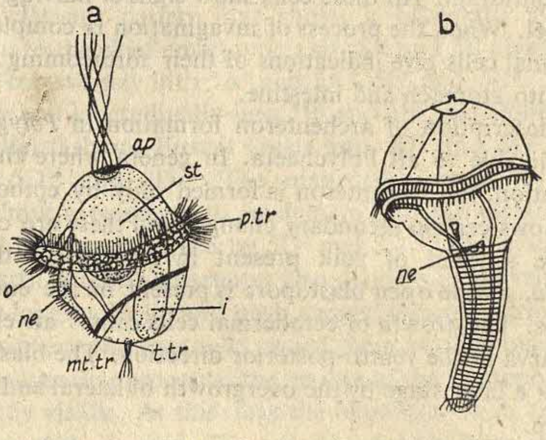


Fig. 9.5. Exo-larval type of metamorphosis in *Polygordius*.
a—Trochophore larva of Archiannelida.
b—Trochophore larva beginning to form trunk segments.
ap—apical plate; i—intestine; mt. tr.—metatroch; ne—protonephridium; o—mouth: p.tr.—prototroch; st—stomach; t.tr.—telotroch.

transformation brought about by the invagination or epiboly of gastrulation. The mouth becomes located just below the ventral edge of the prototrochal girdle, and the anus at the opposite side from the organ, while the digestive tract connecting them is bent in a right angle.

The cilia surrounding the anus develop into telotroch.

With further development the prototrochophore develops into the metatrochophore. In the episphere, eye-spots become differentiated and also radial nerves grow out of the apical organ. In the lower hemisphere, the rudiment of the trunk develops together with the growth of the ventral plate. The swimming capacity of the larva is increased with the development of another ciliary band the *metatroch*. The other changes that are brought about are the development of mesenchymal cells, protonephridia and the clear differentiation of the digestive tract into oesophagus, stomach and intestine. The trochophore larva of *Polygordius* was first discovered by Loven in 1842 and it is also sometimes called as Loven's larva (Fig. 9.5). The appearance of the above-mentioned organs differ from species to species.

Polychate Trochophore

The time of appearance of larval organs is influenced by the amount of yolk. The less amount of yolk in the eggs of any species, the earlier is the stage in which the larval organs appear and hatching takes place. The longer the swimming period, the more schematic is the organization of the trochophore. The more the yolk in the eggs, the trochophore begins its bottom dwelling life immediately after hatching and it will form a degenerate trochophore.

The prototrochophores of Polychaeta can be divided into *monotrochal*, *atrochal* and *mesotrochal* types of larvae according to the mode of formation of the ciliated bands. The monotrochal larva is similar to the archiannelidan larva which was already described, with a single prototrochal girdle encircling the central part of the body. In most cases the formation of the prototrochal girdle is followed by the appearance of the apical tuft and the telotroch. In species where the development takes place in a cocoon, all these organs may not be fully formed but a weak ciliary band corresponding to the prototrochal girdle can always be recognized.

The atrochal larva is the trochophore where all the ciliated bands have degenerated completely. In such larvae the apical cilia are distinctly present, but no ciliary bands are present. However, the embryo is thickly covered with cilia at the time of hatching and give the external appearance of the planula larva of the coelenterates. Atrochal larvae are known in species belonging to the genera, *Sternaspise*, *Lumbriconereis* and *Morphysa*.

In the mesotrochal larva, both prototroch and telotroch have degenerated and a single band of cilia which corresponds to the metatroch develops in an exaggerated form as though to compensate for the missing prototroch. This type of larva is found in the species of *Chaetopterus*, *Telepsanus* and related genera.

The monotrochal type of prototrochophore add to its lower hemisphere, body segments produced by the growth of the ventral plate and the mesodermal bands, and thereby advances to the metatrochophore stage. At this time the metatrochal bands also increase and the larva develops into the standard type of this stage, the *Polytrochal* larva. Depending on the extent of the development of metatrochal bands polytrochal larvae may be classified into three groups: (1) The amphitrochal larva: on each segment a metatroch which forms a complete ciliated band encircling the segment parallel to the prototroch, (2) In the *gastrotrochal* larva, the metatroch fails to extend around the dorsal sides of the segments, forming a band of weak cilia on the ventral side only; and (3) The *nototrochal* larva, the opposite is the case, the cilia forming only dorsally.

The amphitrochal larva represents the basic form of polytrochal larva. There are relatively few cases in which a complete ciliated band is found on each of the segments formed during the metatrochophore stage. More often vestiges of degenerated metatrochs appear as row of weak cilia on the ventral surfaces of each segment.

Cases are known in which closely related species of the same family and genus, produce entirely different types of the polytrochal larva when their ecological habits are different. For example, among the Terebellidae (*Terbella conchilega)*, the larva has a typical nototrochal stage and is free swimming. The larva of *Terebella meckelli* almost completely lacks a free-swimming stage, is said not to produce any metatrochal cilia.

Except in a few species, in which the free-swimming stage is especially pronounced, there is a tendency in the polytrochal larvae of most polychaetes for the metatrochal cilia to degenerate. At the two sides of each body segment the rudiments of parapodia are formed, and numerous paddle-shaped swimming setae or metachaetae appear.

After this stage, the larva gradually begins to exhibit some peculiar polychaetan characteristics, although in most species the free-swimming habit continues. The larva in this period is known as the nectochaetal larva or polychaete larva.

The tufts of swimming setae which characterise this larva help swimming as well as in maintaining equilibrium and rising and sinking. The shapes of these larvae show a great degree of diversity, which is probably the result of a high degree of ecological adaption originating in the mode of development of the setae. In the nereid genera, *Nereis* and *Tylorrhynchus* three paired tufts of setae begin to be formed as the larva proceeds towards the polytrochal stage, and it finally comes to possess the characteristics of a full-fledged nectochaeta. As development continues new segments are added to its posterior end and the tufts of setae also increase accordingly.

In *Arenicola*, the swimming setae are poorly developed and the larva spends part of its time creeping and part of the time swimming. The larva of *Morphysa* passes through an atrochal stage and develops directly into a peculiar nectochaeta (Fig. 9.6).

In some nectochaetal larvae free-swimming habit is highly evolved. In these larvae the swimming setae are strongly developed so that they present a spectacular appearance. This type can be found in the *Polynoe-larva* and *Spio-larva* (Fig. 9.6). In the *Spio-larva*, numerous powerful swimming setae which are longer than the entire length of the body are formed dorsally on both sides of the anterior segments close to the prototroch, so that they completely overlap the larval body. The larvae of the deep-sea polychaete, *Chaetosphaera* is well known as the most conspicuous example of such structure (Fig. 9.6).

The variations in the shapes of these nectochaetal larvae can be found arising within a single species in response to differences

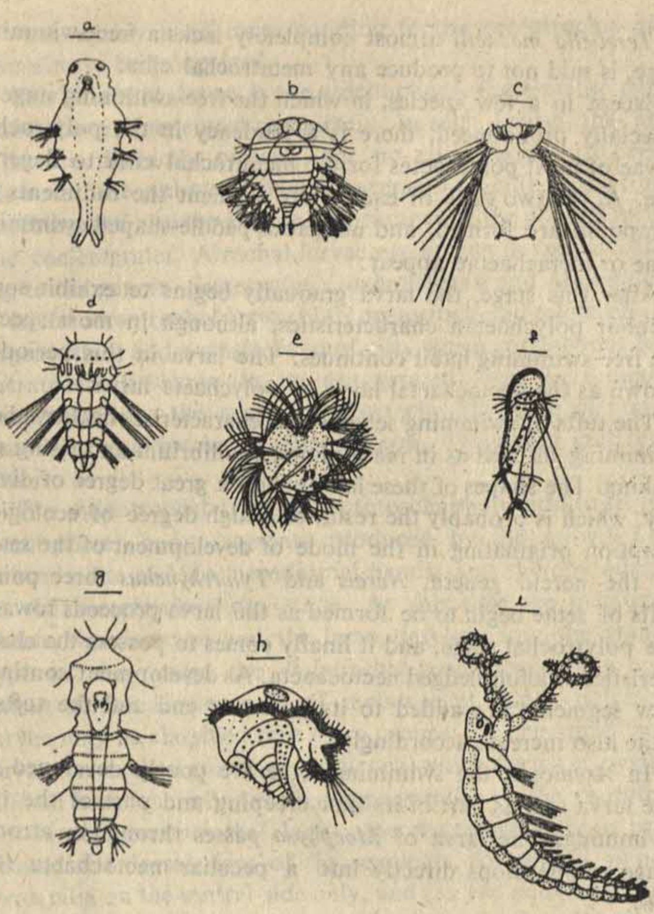


Fig. 9.6. Nectochaetal larvae of Polychaeta.

a—Morphysa.
b—Polynoe.
c—Spio.
d—Polydora.
e—Chaetosphaera.
f—Myzostoma.
g—Ophryotrocha.
h—Mitrarica.
i—Rostraria type larva.

in ecological conditions. In *Nereis dumerilii* pelagic and sedentary individuals are found. The pelagic individuals produce pelagic larvae with well-developed swimming setae, while the sedentary larvae have poorly developed setae.

Mesoderm formation

The most important progress made during the trochophore stage is the formation of mesoderm. It consists of larval mesoblast and the mesodermal bands. The mesoblast arises chiefly from the blastomere of the third quartette. These cells, which have slipped into the blastocoel at the time of gastrulation, take up scattered positions within the cavity and eventually develop into the retractor muscle cells (Fig. 9.7). Throughout the trochophore stage these muscles enable the larval body and especially the digestive tract to expand and contract.

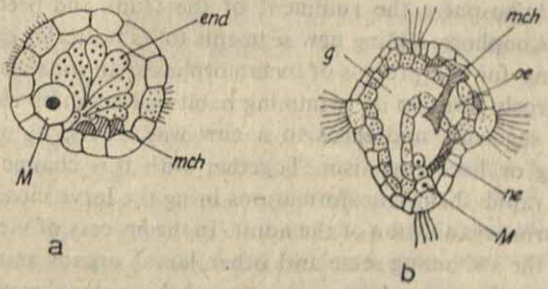


Fig. 9.7. *Hydroides*.
a—Gastrula.
b—Trochophore.
end—endoderm; g—gut; m—mesoblast; mch—mesenchyme cell; oe—oesophagus; ne—nephridiun.

The mesodermal bands arise from the mesoblast cells 4d, and M will develop into the mesoderm of the future adult. This cell lying at the posterior end of the endodermal region at the time of gastrulation divides equally to form the teloblasts of the mesodermal bands. These teloblasts undergo two unequal divisions and the two pairs of micromeres thus formed are left in the endodermal region while the teloblasts themselves gradually move away (Fig. 9.7). Before the larva enters the prototrochophore stage, these M cells have become completely transported into the blastocoel (Fig. 9.7). There they divide repeatedly to form the mesodermal bands, the paired lines of cells which proliferate ventrolaterally along the digestive tract (Fig. 9.7). At first the mesodermal bands grow out in a direction roughly parallel to the prototroch, but after the turning of the larval axis which accompanies the development of the trochophore, they take an anterioposterior position intersecting the prototroch (Fig. 9.7). In the polytrochal larva, the proliferation of mesoderm cells increases still more in response to the growth of the ventral plate. By the time the larva acquires swimming setae and reaches the nectochaetal stage, the bands are segmenting into mesodermal somites which show the beginnings of coelom formation.

Metamorphosis

The free-swimming prototrochophore larva presently forms on its hyposphere the rudiment of the trunk and becomes a metatrochophore, adding new segments to its posterior end and preparing for the process of metamorphosis. In most cases the larva largely gives up its swimming habit and acquires as many as ten segments and turns to a new way of life as either a creeping or fixed organism. Together with this change in its habits, rapid shape transformations bring the larva close to the vermiform organization of the adult. In the process of metamorphosis, the swimming setae and other larval organs retrogress, the trochophore episphere contracts and the tentacular cirri and branchial filaments develop; in the meantime, the trunk segments which have begun to extend posteriorly undergo rapid enlargement and growth.

Metamorphosis of Archiannelidan Trochophore

Two types of metamorphosis, exo-larval and endo-larval are found in this group. The exo-larval type of development was described by Hatscheck and others as it is found in *Polygordius neopolitanus*. In the trochophore of this species, the posterior end of the episphere projects as a trunk with a segmented mesodermal band, the growth of which brings the larva to the beginning of metamorphosis (Fig. 9.5). The fully developed prototroch functions actively enabling the larva to continue free-swimming life for some time. During this period it assumes a vertical posture, with its steadily elongating trunk dangling downward (Fig. 9.5). When there are about 30 segments, the episphere gets thickened. This gradually gives rise to shrunken, cone-shaped preoral lobe, and the prototroch gradually degenerates. As metamorphosis reaches completion, the trunk rapidly increases its number of segments and the young worm gets prepared for a bottom creeping habit (Fig. 9.5). This type of exo-larval metamorphosis is found in many polychaetes.

Endo-larval metamorphosis was discovered by Woltereck, Solensky and Soderstrom in the North sea *Polygordius appendiculatus* and the Black sea form *P. ponticus*. In this case, from the beginning the extension of the trunk takes place not toward the outside as in the exo-larval type, but folded inward from a point at the posterior side of the episphere until ten trunk segments have been formed. However, finally these segments are everted to the outside as in the exo-larva and metamorphosis is completed in the same manner. Metamorphosis among the Mitraria-type larvae is believed to follow this mode almost exactly (Fig. 9.6).

Metamorphosis of the Nectochaetal Larva

The nectochaetal larvae of the polychaete undergo metamorphosis by various processes which are peculiar to each species. In general the nectochaetal larvae have a high grade of adaptation for swimming habit by having strongly developed ciliary bands and powerful swimming setae. Hence the morphological change accompanying metamorphosis take place gradually. In the forms where the adult assumes a sedentary habit after metamorphosis take less time for metamorphosis.

Nereis pelagica is adapted to a pelagic life whereas *Branchiomma vesiculosum* is adapted for a sedentary life. In the following paragraph the concrete changes involved in these modes of metamorphosis will be compared.

The larva of *Nereis pelagica* reaches a typical nectochaetal stage several days after fertilization. This larva has a pair of eye spots and an acrotroch in its head region and behind the prototroch are three segments provided with tufts and a telotroch. In the third week after fertilization, these ciliary bands begin to retrogress in regular sequence and in their place a pair of first tentacles are formed at the anterior tip of the head and paired second tentacles at the side of the mouth. The caudal cirri are located at the posterior end of the trunk (Fig. 9.8). From this time a progressive enlargement and extension of the trunk sets in, and the larval course of development deliberately

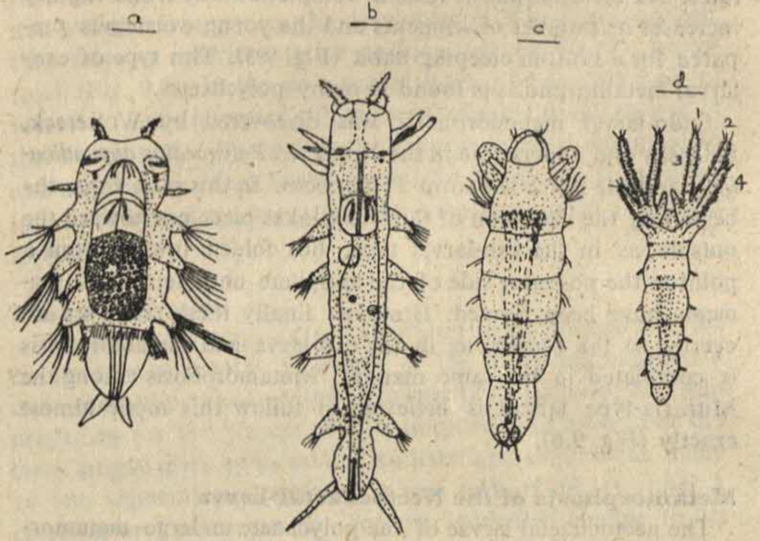


Fig. 9.8. Metamorphosis of nectochaetal larva.
a—Larva of *Nereis pelagica* 18 days after fertilization.
b—Larva of *N. pelagica* 23 days after fertilization.
c—Larva of *Branchiomma vesiculosum* 9 days after fertilization.
d—Larva of *B. vesiculosum* 20 days after fertilization.

turns in a direction appropriate to a creeping existence. By the fourth week, a fourth segment having parapodia and setae appears at the posterior end of the trunk. In the meantime, the setae of the first segment have degenerated and its parapodia have developed into the third pair of tentacles. This constitutes the process of metamorphosis. With further development, segments are added to the posterior end in regular order and the growing worm acquires the vermiform shape of the adult.

The nectochaetal larva of *Branchiomma vesiculosum* due to its sedentary habit has poorly developed ciliary bands and swimming setae. In the second week after fertilization, it forms a pair of eye spots and bulging rudiments of the branchial filaments in the head region. It produces three or four trunk segments and becomes a larva closely similar to the *Rostraria* type. This soon arrives at the stage of metamorphosis and while it is secreting a mucous tube and becoming attached to the bottom, it completes the process of metamorphosis within a few days. By the third week, the head region, which has lost its eye spots, ciliary bands and other larval organs, shrinks to a proboscis-like prostomium, the branchial filament rudiments elongate anteriorly and divide into feathery branches and the trunk extends its growth posteriorward to give rise to the final form of the tubicolous polychaete (Fig. 9.8).

Experimental Embryology

The developmental potentials of an egg can be investigated by experimentation. For this purpose annelid eggs have been used quite frequently. These eggs have been fused, fragmented, centrifuged, compressed or disarticulated, the aim being that of discovering the morphogenetic value of the visible or invisible plasma.

Hatt (1931) fused two eggs of *Sabellaria* before segmentation. The result was not the formation of a single gigantic embryo but of double or multiple embryos. The egg of *Nereis* was fragmented into two parts by Costello (1940) shortly before the first cleavage (Fig. 9.9). If the egg is cut across the equator one

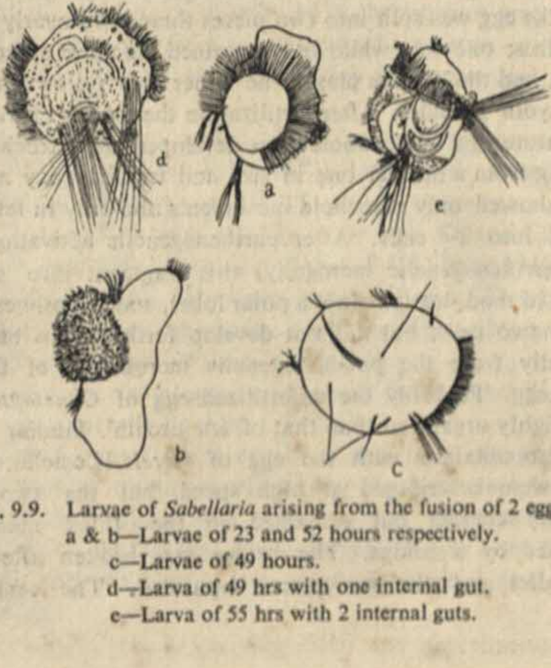


Fig. 9.9. Larvae of *Sabellaria* arising from the fusion of 2 eggs.
a & b—Larvae of 23 and 52 hours respectively.
c—Larvae of 49 hours.
d—Larva of 49 hrs with one internal gut.
e—Larva of 55 hrs with 2 internal guts.

obtains an animal fragment which contains the cleavage spindle and a vegetal fragment which contains no nuclear material. The latter does not develop. The animal fragment, however, cleaves and can also give rise to an essentially normal embryo. This means that before segmentation the egg is omnipotent.

The plasms can be completely displaced or disarranged by centrifugation. Afterwards when the egg cleaves, the plasms can be segregated in unusual blastomeres so that their formative value can be tested. These experiments have been carried out in *Chaetopterus* by Lillie (1906, 1909) Morgan (1910) and Raven (1938).

The first cleavage which falls through the poles gives rise to two unequal blastomeres, the smaller of which contains every possible kind of substance (oils, yolk etc); the polar lobe, which appears at the usual times, may also contain all these materials (Raven, 1938). It is really unexpected to obtain normal larvae from such disarranged eggs.

Wilson (1929, 30) and Harvey (1939), experimented with the eggs by submitting them to stronger centrifugation. As a result of this the eggs were broken into fragments. In the cases in which the egg was split into two pieces these were nearly of the same sizes; one was white and contained the polar spindle, the oil cap, and the hyaline plasm, the other was yellow and contained yolk granules. After fertilization the former cleaved like a miniature egg, and in some cases developed into a trochophore differing from a normal one in size and transparency and the latter showed only amoeboid movements and only in few cases divided into 2-4 cells. After parthenogenetic activation with KCl (parthenogenetic merogony) this fragment also showed amoeboid modifications (not a polar lobe), and sometimes cleaved into two cells, but did not develop further, thus behaving differently from the parthenogenetic merogonies of the sea urchin egg. 'Probably the unfertilized egg of *Chaetopterus* is more highly organized than that of sea urchin'. Similar results were also obtained with the egg of *Nereis* (Costello, 1940). These were centrifuged at high speed, but the two parts did not separate but remained in the thick membrane connected by a bridge. The bridge was broken afterwards by needles and the two pieces separated. The centrifugal fragment, which contain yolk, the material of the cortical granules and some erythrophilic granules, does not cleave at all after fertilization. The spermatozoon is present but does not seem able to stimulate the formation of an aster in the cytoplasm. Costello maintains that it is unable to do so because it requires conditioning by the nuclear sap. The nucleated fragment (containing the oils and the clear plasm), on the other hand, develops in same way. If the anucleated fragment is activated parthenogenetically with KCl it does not show any development, but only amoeboid movement.

Centrifugation has also been carried out on the eggs of *Tubifex* (Parseval, 1922) and *Sabellaria* (Fauri-Fremiet 1924; Haxin 1935; Raven et al., 1950). In *Tubifex* the polar plasms can be completely displaced, with the result that these eggs never develop into normal larvae. After centrifugation of the unfertilized *Sabellaria* egg the ooplasms are distributed as follows: The strongly refingent oil droplets at the animal pole, the heavy yolk granules at the vegetal pole and the hyaline cytoplasm in the intermediate region. Once fertilized, the eggs develop, and the ooplasm is distributed in the arbitrary way in the different regions; sometimes the oils were found even in the polar lobe, which means that its formation is independent of its content.

If *Chaetopterus* eggs are submitted to compression between two plates (Titlebaum 1928; Tyler 1930), they divide equally instead of cleaving into two unequal cells and as a consequence, the vegetal plasm is distributed equally in the cells AB and CD. These eggs develop into double larvae (Fig. 9.10) (Tyler 1930). These are perfectly normal. The blastomeres AB and CD were previously separated. Similar results have also been obtained with the eggs of *Nereis* (Wilson 1896; Morgan 1920); the embryos that develop from eggs compressed before the first segmentation are generally double.

Double embryos from a single egg have also been obtained in *Tubifex* following treatment of the egg by heat, lack of oxygen, etc. (Pennera 1922, 1924 a, b, c, 1926, 1936, 1938). This result is due to the fact that the polar plasms are equally distributed into the two blastomeres which are formed at the first segmentation.

Another important result deriving from the experimental

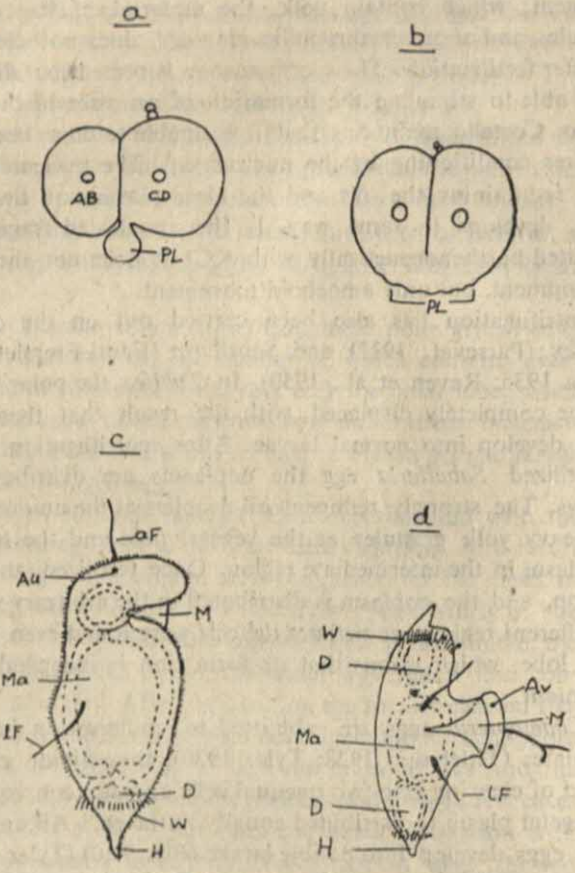


Fig. 9.10. Egg of *Chaetopterus*.
a—Normal 2-cell stage with polar lobe.
b—Egg which segmented into two equal blastomeres.
c—Normal trochophore.
d—Trochophore derived from (b).

investigations on the annelid egg is that concerning the possibility of differentiation without cleavage. This result has been obtained by Lillie (1902) with the egg of *Chaetopterus*. The egg whether fertilized or unfertilized, if exposed for a certain time to treatment with KCl and afterwards returned to normal sea water, fails to cleave. The egg forms the polar lobes normally, the ectoplasm extends over the endoplasm as in a normal larva and a unicellular gastrula is formed. Later the ectoplasm becomes vacuolated as in a normal larva and develops cilia; so an acellular larva, similar to a normal one is formed. It does not have the long apical tuft, nor the trochus, but moves actively in the water. The extraordinary fact made evident by Lillie (1966) is that the capacity to develop the specific ground plane of the embryo is present in the egg before segmentation and that this capacity can materialize up to a certain plane without the intervention of segmentation (Weiss 1939).

The Developmental Capacities of the Isolated Blastomeres

Lot of work on these lines was done by Wilson (1904) on the Lanice egg. The blastomeres, when isolated, cleave as if still part of the entire egg. CD gives rise to a rather normal trochophore (with prototrochus, apical organ, eye and post-trochal region); AB, on the other hand, yields only a partial larva (which has prototrochus and apical organ) but neither eye nor post-trochal region. Similar results have been obtained with the eggs of *Sabellaria* (Hatt 1932: Novikoff 1938) (Fig. 9.12). AB cleaves without forming polar lobes, finally giving rise to a spherical larva which lacks the apical tuft and the post-trochal bristles but which has however, the prototrochal and the apical cilia. CD on the other hand, forms the polar lobes and gives rise to a normal larva (although the cilia and tuft are disproportionately large).

Interesting results were obtained by Costello (1945) with eggs of *Nereis*. The blastomeres were isolated after the eggs had been deprived of their membranes (with alkaline NaCl). They continued to cleave as if still forming part of the embryo, as is shown in Fig. 9.14 where the results of isolation of single cells are shown at the 16-cells stage. The development is a true 'mosaic work', with neither interaction between the blastomeres nor induction ever being observed.

The results obtained by Penners (1922, 1924, a, b, c, 1926, 1936, 1938) with the egg of *Tubifex* are also very interesting. In these experiments the blastomeres were not isolated, but some were killed with ultraviolet radiation and the development of the remainder was studied (Fig. 9.13). The results were as follows:

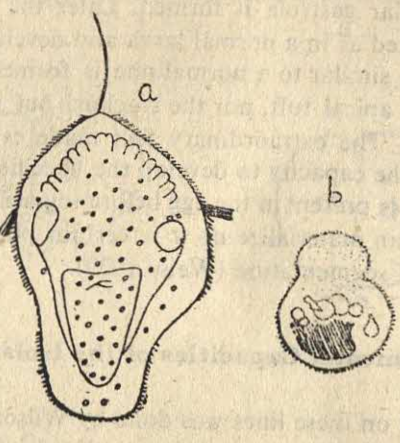


Fig. 9.11. *Chaetopterus.*
a—Normal trochophore.
b—Acellular trochophore derived from an activated but not segmented egg.

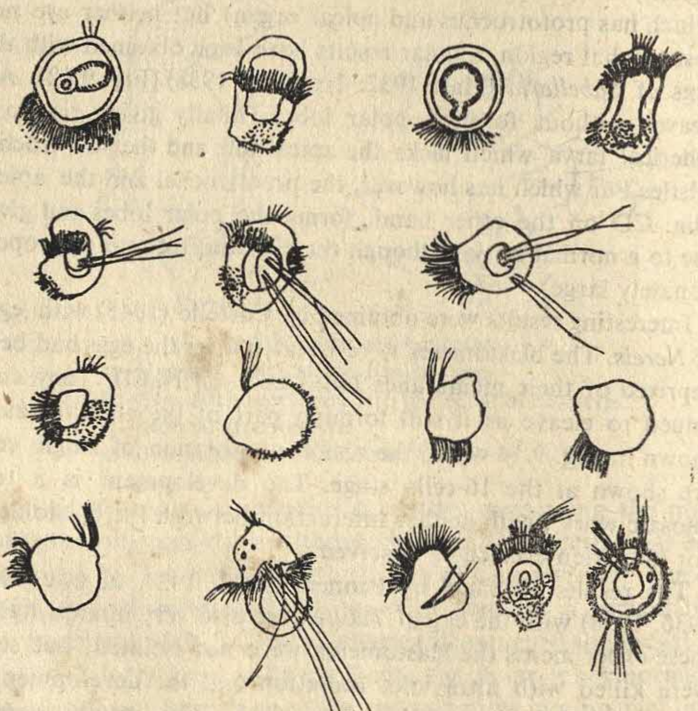


Fig. 9.12. Larvae from isolated blastomeres.

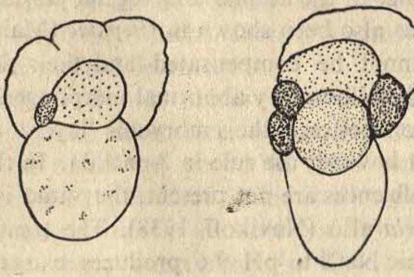


Fig. 9.13. Development of an egg of *Tubifex* after removal of A, B, C cells.

1) Development of the isolated D-cell (A.B.C. killed): continues to develop as if still in the usual conditions, giving rise to the 1st and 2nd somatoblasts and finally to a small perfectly proportioned animal. This result shows that the D-cell, which ordinarily would have formed only a part of the embryo, is capable by 'regulation' of giving rise to an entire embryo.

2) Development of A+B+C: only formless and undifferentiated masses of ectoderm and endoderm result from the development of these blastomeres.

3) Development of the egg from which the 2d- and 4d-cells were removed: the germ bands do not form and the embryo is similar to that obtained in (2);

4) Development of the egg without the 2d-cells: the remaining cells continue to divide and give rise to the second somatoblast (4d), Myr and Myl and the mesodermic bands. The embryo as finally formed, lacks the organs deriving from the ectodermic bands. A regulation however is observed; embryos reared for a long time do form the nervous system. It can be shown that its formation is due to a regulation arising from the mesodermic derivations (Penners 1936).

5) Development without 4d: the remaining cells continue to develop typically; they give rise to the first somatoblasts ($2d^{111}$) and afterwards to its derivates (Nr and Mr).

At the end of development, however, the embryo lacks all the organs which derive from the mesodermic bands, the organs which derive from the mesodermic bands are also irregularly disposed. That means that the territories deriving from 4d exert an influence on the territories deriving from 2d.

The importance of the animal and vegetal plasms for normal development has also been shown in *Clepsine* (Maie 1932), their elimination cannot be compensated and their dislocation by centrifugation is followed by abnormal morphogenesis.

An interaction between the embryonic layers as shown in *Tubifex*, is not however the rule in Annelida. In the *Nereis* egg such mutual influences are not present, the same is true of the egg of *Sabellaria* also (Novikoff, 1938). The treatment of this egg with isotonic NaCl to pH 9.6 produces exogastrulation so that the endoderm remains on the exterior and the ectoderm does not enter into contact with it (Fig. 9.14).

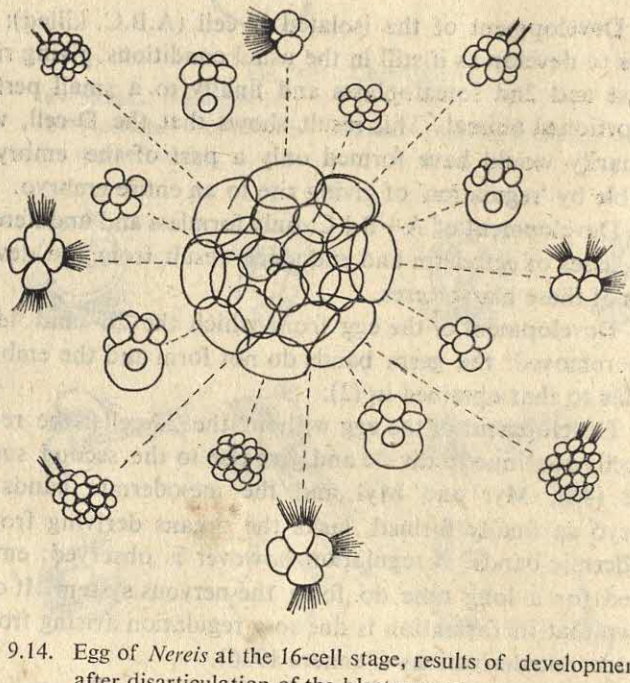


Fig. 9.14. Egg of *Nereis* at the 16-cell stage, results of development after disarticulation of the blastomeres.

Biochemical Investigations

This field which should constitute the ultimate step in embryological research has been scarcely cultivated; probably for technical reasons. Weber (1958) used the eggs of *Tubifex*, the relatively large size of the egg enables the biochemical research to be carried out. Weber proposed to ascertain whether the different blastomeres of the developing egg have a different metabolism and eventually whether there is a causative relation between metabolism and differentiation.

The activity of cytochrome oxidase and cathepsin in 2d-4d cells (somatoblasta) and macromeres was considered. The activity of cytochrome oxidase in second 4d (Nadi-positive somatoblasts) is three times higher than in the Nadi-negative macromeres: this result agrees with the data obtained by cytochemistry and electron microscopy concerning the distribution of the mitochondria. The activity of cathepsin, on the other hand is lower in 2d and 4d than in the macromeres. A new line of research is that of Winesdonfer (1965 b) who studied the amino acid incorporation in the postmitochondrial supernatants of the unfertilized egg of *Sabellaria*.

Early Development of the Oligochaeta

In this group, the entire development from fertilization to the time of hatching takes place within the protective cocoon. In the basic aspects the development of oligochaetes resembles that of the polychaetes but there is no trace whatsoever in oligochaete development of a free-swimming stage with a prototroch. After the gastrula stage, the proliferative growth of the ectoderm cells produce the trunk rudiment, and growth proceeds step-by-step in a direct fashion towards the adult vermiform morphology, without the complicated process of metamorphosis seen in the Polychaeta. Studies on the embryology of the oligochaetes shows that the direct mode of development is characteristic of not only terrestrial and freshwater oligochaetes but of the marine forms as well.

The amount of yolk contained in the eggs of oligochaetes varies with the species. Generally the eggs of aquatic forms are large and yolky, while the terrestrial forms have small eggs with little yolk. One striking feature of oligochaete egg cells is the inclusion of a so-called pole-plasm. The egg cytoplasm consists of an opaque, yolky portion, and a clearly different active protoplasm. This material is about evenly distributed throughout the immature oocyte, but when polarization of the egg substance takes place at the time of polar body extrusion, it collects in separate masses, near the animal and vegetal poles.

For this reason these masses of active protoplasm located at the two poles of the mature egg are called poleplasm.

As in Polychaeta the mode of cleavage is basically total, unequal and spiral. A comparison however shows, that some of the conspicuous blastomeres in polychaete development play a less striking role in the oligochaetes and vice versa. The degree of size inequality among oligochaete-blastomeres is so marked from the earliest divisions that at first glance the cleavage appears to be less spiral than irregular. The following account of cleavage of oligochaetes is based on Panner's (1922) description of *Tubifex rivulorum*.

The first cleavage of *T. rivulorum* is unequal and the two masses of pole-plasm are contained in the large CD blastomere. In the second cleavage, CD divides unequally into a small C and a large D blastomere, while AB cleaves equally into A and B to produce the four-cell stage. The third cleavage is an equatorial division and gives rise to an eight-cell stage which includes the first quartette of micromeres; the blastomere 1D is especially large and contains both pole-plasms.

The cleavage of these blastomere group is spiral. The third cleavage like in polychaetes is dexiotropic and the fourth cleavage laeotropic. Due to inequality, the synchrony of cleavage is already disturbed at this fourth division, so that 1D and 1C separate off their second quartette micromere before 1A and 1B giving rise to a 10-cell stage.

At this cleavage, for the first time, the animal and vegetal pole-plasms are separated into different blastomeres. The 2d blastomere is much larger and includes the animal pole-plasm and this cell is destined to be the first somatoblast. The 2D blastomere contains the vegetal pole-plasm and yolk.

After the 10-cell stage, the synchrony of cleavage of the various quartettes becomes still more disrupted giving rise to a divergence in the time of micromere formation of the several quadrants. As a consequence, the division process fails to follow the typical spiral course with 16-cell and 82-cell stages. In general the D quadrant divides first followed by the C quadrant. The 22-cell stage follows the 10 cells. It contains four yolky macromeres (3A, 3B, 4C and 4D) in the vegetal hemisphere and in the upper half, the two large blastomeres $2d^{11}$ (first somatoblast) and 4d (second somatoblast) as well as

16 micromeres. The 4d blastomere is more posterior, contains no yolk, includes most of the vegetal pole-plasm and destined to be the mesoderm mother cell. The first and second somatoblasts, having the two pole-plasms play an important role in the ensuing development of the embryo.

The 16 micromeres which occupy the rest of the upper hemisphere lie interposed between the first somatoblast and the four macromeres of the lower hemisphere, forming a micromere band which fills this space. Shortly afterwards an extremely limited blastocoel develops in the centre of the blastula. Among the terrestrial oligochaetes, the eggs of which have less yolk, the inequality between the blastomeres is somewhat less extreme, and the embryo formed as a result of cleavage is correspondingly closer to the coeloblastula.

Germ Layer Formation

In the yolk-laden aquatic oligochaete eggs, gastrulation takes place chiefly by epiboly. In the eggs with less yolk, gastrulation is by invagination. The eggs of the marine oligochaete, *Pachydrilus leneatus* contain large amount of yolk. In the blastula of this species, ectodermal bands formed by the proliferation of the first somatoblast descendants develop prominently on each side before the endodermal cell group has completed formation of the archenteron; these cellular bands undergo marked growth and envelope the endodermal cells from both sides, bringing about closure of the blastopore (Fig. 9.15).

When the eggs have little yolk as in *Lumbricus*, the embryo has a rather well-developed blastocoel and the archenteron is formed by the invagination of the blastomeres located in the vegetal hemisphere. In this case also the ectodermal bands attain a conspicuous growth, so that the external appearance is not very different from that of the gastrula formed by epiboly. In either case the ciliary bands which characterize the trochophore larva have completely degenerated and fail to appear. The striking development of the ectodermal and mesodermal bands make it easy to distinguish among the three differentiating germ layers. The ectodermal bands arise from the first somatoblast, by the gastrula stage it has increased to become four conspicuous rows of cells on each side of the embryo

(Fig. 9.15). By the blastula stage this first somatoblast 2d has divided repeatedly, $2d^{11}$ being formed from $2d^1$ and then $2d^{111}$, this $2d^{111}$ for the first time undergoes equal cleavage, giving rise to the left and right teloblast T^l and T^r. During the progress of the embryo from blastula to gastrula, these teloblasts divide in a special fashion, eventually producing on each side the four teloblasts N, m^1, m^2 and m^3. These form the starting points from which the cells proliferate on each side to give rise to the cellular bands. The latter occupy the animal hemisphere and together with the other micromere quartettes comprise the ectoderm.

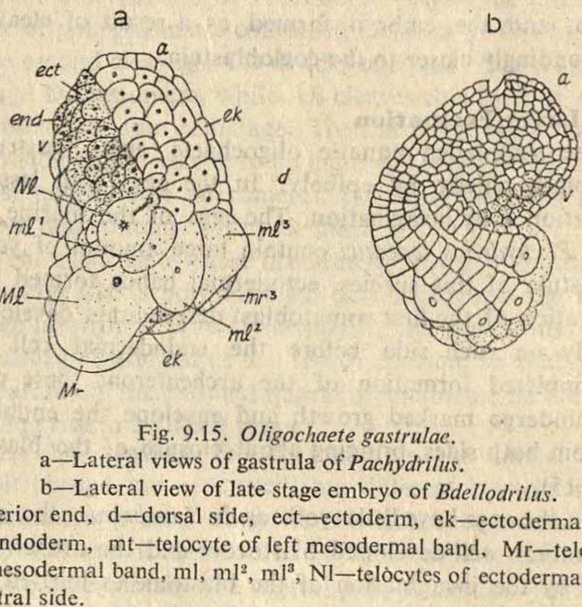


Fig. 9.15. *Oligochaete gastrulae.*

a—Lateral views of gastrula of *Pachydrilus.*

b—Lateral view of late stage embryo of *Bdellodrilus.*

a—anterior end, d—dorsal side, ect—ectoderm, ek—ectodermal band, end—endoderm, mt—telocyte of left mesodermal band, Mr—telocyte of right mesodermal band, ml, ml^2, ml^3, Nl—telocytes of ectodermal band, v—ventral side.

The second somatoblast 4d gives rise to the mesodermal bands. This blastomere divides equally into right and left cells to form on each side the mesoderm mother cells M^r and M^l. Because of the closure of the blastopore by epibolic growth, the cells M^r and M^l, get crowded into the posterior end of the blastocoel, where they proceed to give rise to the mesodermal bands. In this stage it is possible to distinguish an entodermal group at the animal end and an ectodermal group consisting of micromere quartettes and the cellular bands originating from the four teloblasts from each side. The mesoderm is in the form of two bands proliferating bilaterally into the blastocoel from the two teloblast cells.

The continuous proliferation of these cellular bands causes the originally spherical embryo to become first oval and then elongated. The shifting of the embryonic axis which takes place in the polychaete embryo takes place here also on the same principle. Following the vigorous development of the mesoderm, body segments proliferate posteriorly and the embryo gradually acquires a vermiform shape. It shows feeble peristaltic movements within the cocoon and hatches by breaking through the cocoon wall.

Early Development of Hirudinea

The developmental processes in Hirudinea is very similar to that of the Oligochaeta in the embryonic stages. In the leeches the early development takes place directly in a protective cocoon. The following account is based on the rhynchobdellid *Clepsine* and the gnathobdellid, *Nephelia*.

The leech egg also contains animal and vegetal pole-plasm. In principle the mode of cleavage is spiral. It does not follow the regular course as seen in the Polychaeta. The extreme irregularity of the size in the blastomeres completely blocks the synchrony of the division cycles as a result of which further irregularity is found.

In *Clepsine* the cleavage goes on in a typical spiral fashion until the formation of the first micromere quartette (Fig. 9.16). In the following divisions which lead to the 16-cell stage the C and D quadrants show a tendency to precede the others in cleaving to produce the second and third micromere quartettes (Fig. 9.16). The 2d blastomere, as in oligochaetes is separated off as a large cell containing the vegetal pole-plasm and destined to play the role of first somatoblast. After the 16-cell stage, the cleavage synchrony is completely lost. The micromeres and the blastomeres of the D quadrant continue to divide at a rapid rate (Fig. 9.16).

The D quadrant has no connection with the endodermal layer since the endodermal layer is made up of cells derived from 3A, 3B and 3C only. This peculiarity is characteristic of

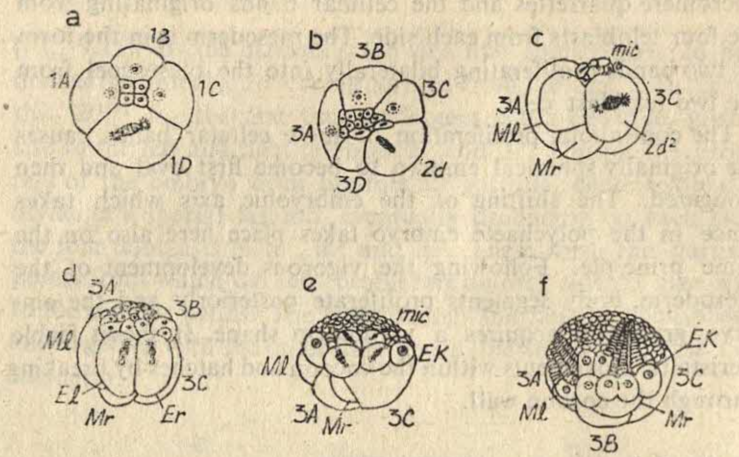


Fig. 9.16. Cleavage in *Clepsine sexoculata*.
Ek—telocyte of ectodermal band.
El—telocyte of left ectodermal band.
Er—telocyte of right ectodermal band.
mic—micromere; ml—left mesoblast;
Mr—right mesoblast.

the genus *Clepsine*. The 3D blastomere includes the vegetal pole-plasm, precociously becomes the second somatoblast. It divides obliquely into equal-sized blastomeres which form the mesoderm mother cells M^r and M^l on the two sides of the embryo (Fig. 9.16). The first somatoblast 2d at once divides unequally, giving rise to a small $2d^1$ and a large $2d^2$, $2d^2$ again divides equally and longitudinally this time to form E^r and E^l, the end cells of the ectodermal bands on the right and left sides (Fig. 9.16). These eventually develop into the ectodermal teloblasts (EK) on each side (Fig. 9.16). The mesoderm mother cells M^l and M^r also lie diagonally at first, but gradually take on a bilaterally symmetrical arrangement (Fig. 9.16).

In the genus *Nephelis* the hirudinean characteristics are more marked than in *Clepsine*. After giving off the first and second micromeres, the blastomeres A and B become yolky and do not divide during the rest of the cleavage period. Most of the cells of the embryonic body, hence are derived from the D blastomere. The 1D blastomere gives off the first somatoblast (2d). This divides continuously and develops into the bilateral teloblasts of the ectodermal bands. The 2D macromere divides twice more forming 3d and 4d. 2c, 3d and 4d, finally make their way into the interior of the embryo, where they are destined to form endoderm together with the yolk cells 1A, 1B and 2C. At this stage the 4D cell containing the vegetal pole-plasm undergoes equal cleavage for the first time, to produce the two mother-cells of the mesoderm (Fig. 9.17).

So, in the leeches the cleavage pattern is fixed at an early stage and each blastomere follows a direct course forward to

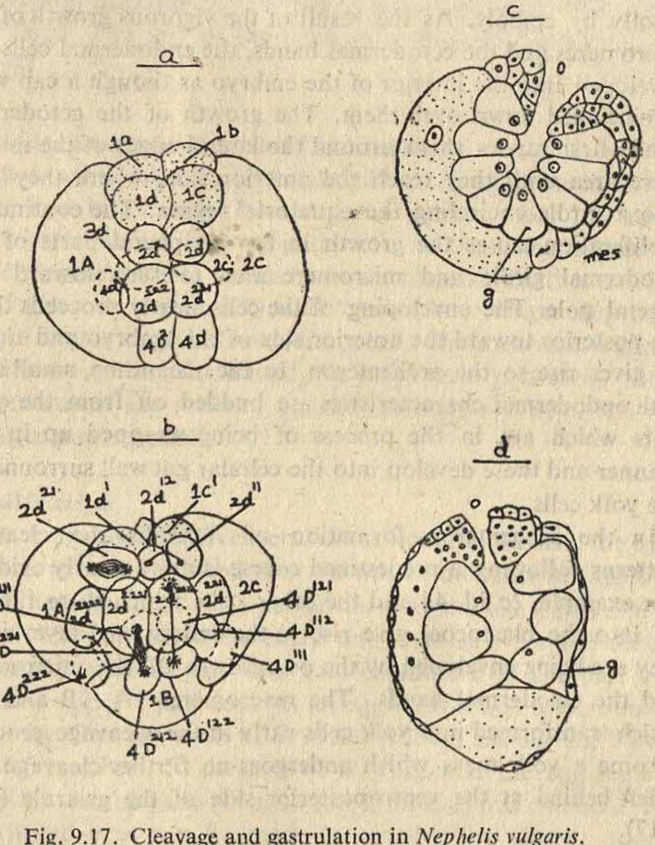


Fig. 9.17. Cleavage and gastrulation in *Nephelis vulgaris*.

a—16 cell stage, from side of D—quadrant.

b—27-cell stage, from side of D-quadrant.

c—l.s. of embryo at stage of stomodaeum formation.

d—More advanced stage.

ect—ectoderm; g—gut; mes—mesodermal band; o—mouth.

its final destiny in an even more pronounced fashion than seen in the oligochaetes. In the leech blastula, the few yolk-containing macromeres in the vegetal hemisphere form the endodermal rudiment, the numerous micromeres present at the animal hemisphere form the ectoderm of the head region. The teloblasts of the ectodermal bands and the mesoderm mother cells, which are present on the two sides at the posterior end of the embryo start to bud off daughter cells toward the anterior end to form the ectodermal and mesodermal bands.

The gastrulation in the genus *Clepsine* is formed almost wholly by epiboly. As the result of the vigorous growth of the micromeres and the ectodermal bands, the endodermal cells are enveloped into the interior of the embryo as though a cap were being pulled down over them. The growth of the ectodermal bands first carries them around the lateral edges of the micromere area until they reach the anterior side, where they fuse into a girdle encircling the equatorial region. The continuous proliferation causes the growth in the peripheral parts of the ectodermal girdle and micromere area to turn toward the vegetal pole. The enveloping of the cells slowly proceeds from the posterior toward the anterior side of the embryo and thereby gives rise to the archenteron. In the meantime, small cells with endodermal characteristics are budded off from the yolk cells which are in the process of being wrapped up in this manner and these develop into the cellular gut wall surrounding the yolk cells.

In the archenteron formation of *Nephelis* the cleavage patterns following a predestined course is more clearly evident. For example, 2c 3d, 4d and the other cells which were thrown off into the blastocoel give rise to the endodermal layer while they are being enveloped by the overgrowth of the micromeres and the ectodermal bands. The macromeres 1A, 1B and 2C, which transformed into yolk cells early in the cleavage process, become a yolk mass which undergoes no further cleavage and is left behind at the ventroposterior side of the gastrula (Fig. 9.17).

In both cases, soon after the endodermal cell layer has enveloped the yolk cells in the blind sac of the archeteron, a small ectodermal invagination, the stomodaeum, forms at the place where the anterior tips of the ectodermal bands have met and fused. This then connects with the archenteron. Once the embryo has reached the gastrula stage, the proliferation of the cells making up the ectodermal and mesodermal bands causes the trunk region to grow, stretching the external form of the embryo into an oval one. The embryo continues to develop and elongate into a vermiform shape of the adult. In many of the species, the embryo begins to shed its egg membrane about this time, and carries on the process of organogenesis within the cocoon, nourished by the albuminous material with which it is filled. The rest of the development takes place inside the cocoon.

Early Development of the Echiuroidea

. These are thick, short worms with an elongate ovoid shape. There is a bifurcate proboscis at the anterior end. No metameric organization and no parapodia in the trunk segments. In development these animals show many similarities to the polychaetes in having a typical trochophore stage. Segments appear at this stage but later disappear. The proboscis is considered to be a modification of the polychaete proboscis. Setae are present at the anterior end of the body and nephridia are arranged metamerically.

Fertilization

Just as in case of Polychaeta, in the Echiuroidea also, the eggs and the sperms are shed into the water and hence fertilization is external. In the genus *Bonellia*, there is marked sexual dimorphism. The male is quite small and lives as a parasite on the female and hence in this case the fertilization is internal. The fertilized eggs are laid down in an egg mass which is surrounded by a jelly. The egg mass of *B. viridis* is long, slender and in the form of a ribbon. In the case of *B. fuliginosa* the egg mass is spherical. In the genus *Urechis* the fertilization is external. The unfertilized eggs can be made to develop by simply adding spermatozoa to them in their sea-water medium. These eggs serve as a valuable material for experimental embryological studies. The following account gives the summary of the fertilization process in the Japanese species, *Urechis unicinctus*.

The spermatozoa of this species measures about 70μ in length, with a round head and long tail. The unfertilized egg measures about 102μ in diameter, usually has one or more indentations. An egg membrane covers the surface, and the cytoplasm is granulated, transparent, with light-yellowish colour. At the centre of the egg there is a germinal vesicle measuring about 60μ and a nucleolus in it.

When the spermatozoan attaches to the egg membrane, a small hyaline fertilization cone forms on the surface of the egg and extends to the membrane. The spermatozoa passes through this process and penetrates the egg surface. As it enters, a sort of deformation appears in the cortical layer of the egg. The surface indentation fills up and about 10-15 minutes after insemination the egg becomes completely spherical.

The germinal vesicle breaks down at this stage and the nucleolus disappears. The egg membrane becomes wavy and elevated and after the egg is completely spherical it becomes reinforced as the fertilization membrane.

Within the perivitelline space numerous radiating filaments appear and the diameter of the fertilized egg is smaller than that of the unfertilized egg. After the fertilization membrane has been formed, a slight polarization of the egg cytoplasm takes place and the first polar body is given out from the somewhat flattened animal pole. The second polar body is formed about 20 minutes later. The sperm pronucleus unites with the egg pronucleus to complete the process of fertilization about 70-80 minutes after insemination.

Cleavage and Germ Layer Formation

The cleavage and the germ layer formation in the echiuroids resembles that of the polychaetes. The eggs of *Urechis* and *Thalassema* cleave almost equally in a spiral fashion. In the genus *Bonellia*, the eggs undergo typical spiral cleavage, with characteristic size differences between macromeres and micromeres. In both cases, the first, second and third quartettes are formed and the fate of each blastomere corresponds exactly to those of the Polychaeta. The group of macromeres which remain after giving rise to the third micromere quartette similarly separate off the mesoderm mother cell in the D quadrant and all become endodermal.

In *Urechis* and *Thalassema* further development leads to the formation of a coeloblastula. The cell proliferation produces a thickening at its vegetal pole, which invaginates to form the archenteron (Fig. 9.18). The mesoderm mother cell is situated at the posterior side of the blastopore, and the mesodermal bands begin to develop with the process of blastopore closure as it progresses from the posterior to the anterior end (Fig. 9.18).

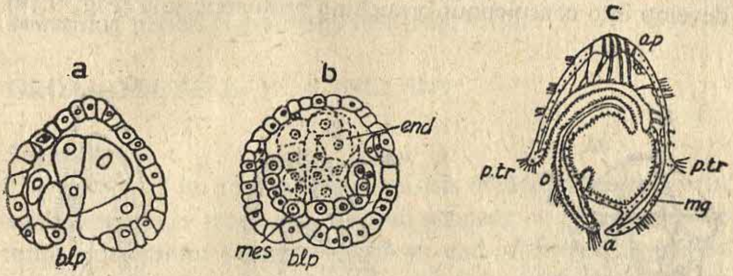


Fig. 9.18. Gastrula and trochophore larva of *Thalassema malitta*.
a—Median l.s. of gastrula at invagination of archenteron.
b—H.S. of gastrula at closure of blastopore.
c—Median l.s. of trochophore larva.
a—anus; ap—apical plate; blp—blastopore; end—endoderm; mes—mesoderm; mg—midgut; o—mouth; p.tr.—prototroch.

In *Bonellia* however, the vegetal macromere group is overgrown by the ectodermal layer and forms the archenteron with the blastopore still wide open. The gastrulae of both types develop apical tufts of cilia and prototrochal girdles and hatch as trochopore larvae.

Larval Stage and Metamorphosis

The young trochophore is completely covered by the cilia and the prototroch stands out as a band of particularly well-developed cilia. There is a prominent ventral plate and conspicuous rows of cilia parallel to the midline on the ventral side. There is a long oesophagus. The midgut is constricted into anterior and posterior chambers. The posterior chamber leads into a short hind-gut which in turn opens to the outside through the anus. The larval mesenchymal cells are scattered throughout the body and form the retractor muscles connecting the digestive tract with the body wall (Figs. 9.18, 9.19). The development of the mesodermal band is very slow but by the time the larva arrives to the height of the free-swimming stage, they show vigorous growth, forming the trunk rudiment and dividing it into mesodermal segments. The rudiments of the protonephiridia can be recognized in the young larva as paired cell masses lying ventrally, anterior to the anus. These are pushed forward by the subsequent growth of the mesodermal bands and develop into conspicuous branching protonephridia (Fig. 9.19).

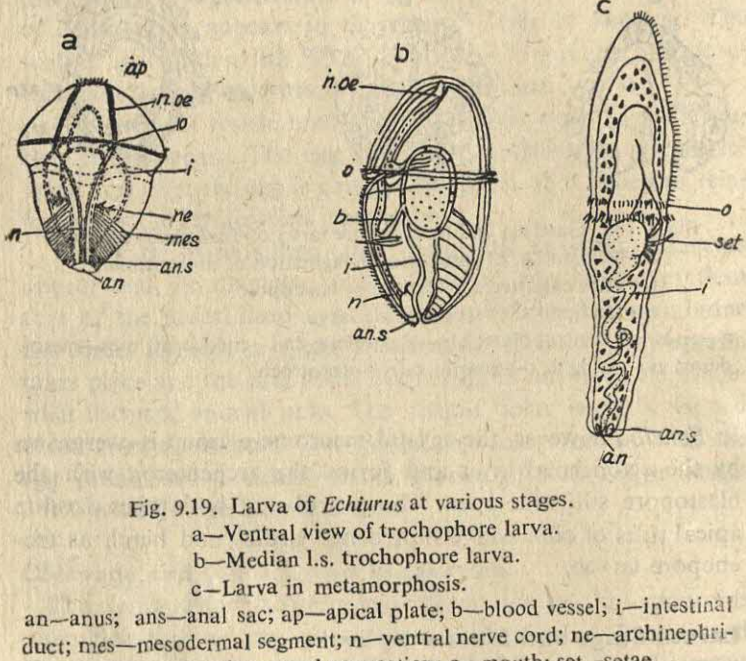


Fig. 9.19. Larva of *Echiurus* at various stages.
a—Ventral view of trochophore larva.
b—Median l.s. trochophore larva.
c—Larva in metamorphosis.
an—anus; ans—anal sac; ap—apical plate; b—blood vessel; i—intestinal duct; mes—mesodermal segment; n—ventral nerve cord; ne—archinephridium; n-oe—circumpharyngeal connection; o—mouth; set—setae.

According to Baltzer, the urechid larva spends one or two months as a trochophore, well adapted to a free-swimming habit, but eventually it undergoes a striking metamorphosis in both external form and internal structure as it turns to bottom dwelling. The metamorphosing larvae are about 1.5 m long, and can often be found in swarms among the bottom layer of plankton.

During metamorphosis, the larva loses its prototroch and increases in length. The curved digestive tract also elongates proportionally. On the surface of the trunk the characteristic urechid papillae are beginning to appear and ventral setae are formed on the anterior end of the ventral surface as well as caudal setae around the anus. After metamorphosis the prostomium grows anteriorly to form the cone-shaped proboscis; the trunk elongates markedly to produce a vermiform body, and the metameric organization which appeared during the free-swimming period is gradually lost (Fig. 9.19).

ORGANOGENESIS IN ANNELIDA

Segments

The body of an adult annelid consists of three principal parts, a prostomium, a trunk and an anal segment or pygidium. The term prostomium refers to the front end of the body, anterior to the mouth. This arises from the episphere of the trochophore stage, which persists in a vestigial form even after metamorphosis. The prostomium gives rise to the tentacular cirri, eye-spots and super-pharyngeal ganglion. Since the mesodermal bands do not extend as far forward as this, the space inside the prostomium is filled with mesenchyme cells, and no free coelom is formed.

The main part of the trunk is made up of a succession of metameric segments. After metamorphosis, these segments are gradually extended posteriorly by the growth of the ectoderm cells. In the fully differentiated segments there is a coelom surrounded by a peritonium, while the posterior part of the trunk consists of an undifferentiated region which will produce new segments.

The pygidium, lying continuous to this undifferentiated segment region, constitutes the last segment, which surrounds the anus. Its central cavity consists of the unexpanded blastocoel remnant and like the prostomium, the pygidium cannot be called a true segment.

The undifferentiated segment which make up the posterior end of the trunk gradually forms new segments anteriorly as the growth of the mesoderm brings about cell proliferation. The segment-producing capacity of this undifferentiated region is in a sense, used up during the course of later development in forming the number of segments highly characteristic of each species, but even in the fully grown adult this part of the body retains a strong, although concealed, capacity for segment formation.

In connection with the process of metamorphosis, the two or three anteriormost segments of the trunk, which characterized the larval stage, lose their setae, change their parapodia into tentacular cirri or branchial filaments, to form a peristomium and together with the prostomium make up the head of the adult worm.

Setae, Setal Sac, Parapodia

The setae are characteristic of Annelida and serve as important taxonomical criteria. As the body segments are formed regularly, regular bud-like setal sac rudiments appear on both sides of the body-wall. These develop into setal sacs by an inward proliferation of their cells and invagination into the mesodermal layer. The setae are formed by a secretion of the cells making up the walls of the setal sacs. As the setae increase in length and thickness they extend outward, developing into various shapes characteristic of each species. The number of setae found among the Polychaeta is extremely large and since they are accumulated into tufts on the parapodia, they present a rather complicated appearance, but their basic principle is actually the same as that of the Oligochaeta. The polychaete parapodium is a stumpy swelling of the body wall at the sides of each segment, in most cases it branches into a dorsal notopodium and a ventral neuropodium. The swimming setae of the larval period show nearly as many shapes as there are species and modes of life. These are lost after metamorphosis, however, and the adult setae make their appearance anew in conjunction with the development of the parapodia, forming dorsal and ventral setal tufts on the notopodia and neuropodia respectively.

Sense Organs and Nervous System

Highly developed sense organs are very few in Polychaeta. The eye-spots and the tentacular tuft of the free-swimming larva disappear after metamorphosis. In their place tentacular cirri are formed after metamorphosis. All these so-called sense organs are developed from the ectoderm.

The process by which the nervous system develops in the Archiannelida is applicable to all the Polychaeta without change. The thickening of the apical plate ectoderm in the episphere of the trochophore larva gives rise to the suprapharyngeal ganglionic mass; the ventral cord rudiment is derived from the neural plate. This in turn develops from the so-called ventral plate which is formed where the two sides of the trunk ectoderm unite ventrally. The anterior end of neural plate forms the sub-pharyngeal ganglion. The two circumpharyngeal connectives are also formed from the anterior end of the neural plate. As development proceeds, the neural plate successively divides—starting at the front end and proceeding backward into segments corresponding to the body segments to produce the ventral nerve cord.

Among the urechids also, the cerebral ganglion rudiment appears in the same way, as an ectodermal thickening of the trochophore apical plate, gives rise to a nerve ring encircling the oesophagus and connects with the ventral nerve cord. This latter arises as a segmented structure along the midline of the ventral plate, but secondarily loses its segmentation (Fig. 9.19).

The development of the nervous system in the Oligochaeta and Hirudinea is rather different from that of the Polychaeta. The larva in these groups do not have organ corresponding to the apical plate but the four cell rows of the ectodermal bands are conspicuously developed. The most ventrally located of these cells correspond to the polychaete neural plate. These cells are called the neuroblasts. The ventral nerve cord connections are formed by the growth of these neuroblasts except that, at least in *Tubifex* and *Lumbricus* the suprapharyngeal ganglion consists of only ventral nerve cord elements.

Epidermal Layer

The cells derived from the micromeres form the epidermis, covering the head region. The ectodermal bands give rise to the rows of neuroblasts, as well as rows of myoblasts which will later become the circular muscles. The remaining cells form the epidermis of the trunk. The ectodermal bands are prominent in oligochaete and leech embryos and the fates of their component cells are well established. Among the four rows of cells which arise at the mid-ventral line on each side of the embryonic body and extend dorsally, the most ventrally located ones become the neuroblast rows (N^l and N^r) and the described above, constitute the rudiment of the ventral nervous system. Of the six remaining rows, the most dorsally located two m^{13} and m^{r}3 are believed to form the setal sacs and the median rows m^{l}1, m^{r}1 and m^{l}2, m^{r}2 mainly develop into the circular muscles of the body wall (Fig. 9.20). There are especially prominent circular muscles around the mouth in the leeches.

Digestive Tract

The ectoderm that is in contact with the mouth invaginates to form the stomodaeum, which connects with the endoderm of the trochophore archenteron to give rise to oesophagus. The endodermal layer itself differentiates into a large sac-like stomach and an intestine which elongates posteriorly as development proceeds. The hindmost end of the intestine in turn, connects with a small ectodermal invagination, the proctodaeum and the anus opens there. The mass of endoderm cells bundled in together by the process of epiboly finally differentiates into a digestive tract, however, a complicated process of cellular fusion and separation takes place. In general the large yolk laden cells lie in the centre of the mass and tend to unite with one another while the small peripheral cells without yolk multiply and draw away from each other, becoming organized into an intestinal wall, which gradually digests and absorbs the yolk cells and finally forms the intestinal tube. The wall of the digestive tract is later surrounded by muscle cells and a peritoneal layer differentiated from the mesoderm and develops into an alimentary tract capable of making chewing and swallowing movements.

Mesodermal Segments

The mesoblasts are clearly evident from a very early developmental stage, as they become the teloblasts of the mesodermal bands on the two sides of the embryo and produce a succession of mesoderm cells. The growth of these bands is a conspicuous characteristic of the annelidan developmental process. As the formation of the trunk progresses, the mesodermal bands begin to form segments one by one from the anterior toward the posterior, separating off a cell mass for each segment, like boxes in a row (Fig. 9.20). These mesodermal cell masses can probably be called somites (Fig. 9.20). Close to the teloblasts, moreover, are segments consisting of still undifferentiated mesodermal band tissue, which continue to grow as the trunk develops.

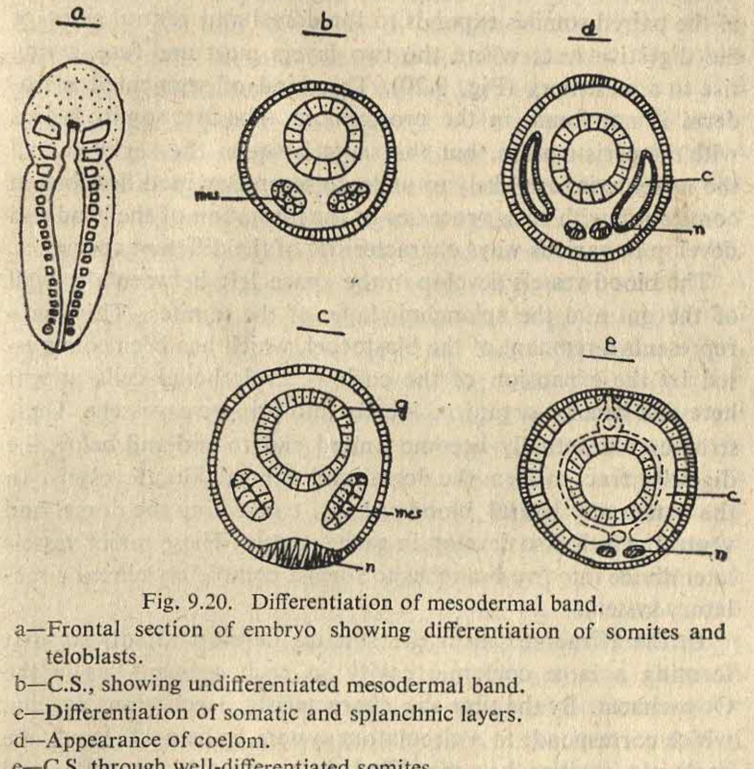


Fig. 9.20. Differentiation of mesodermal band.

a—Frontal section of embryo showing differentiation of somites and teloblasts.

b—C.S., showing undifferentiated mesodermal band.

c—Differentiation of somatic and splanchnic layers.

d—Appearance of coelom.

e—C.S. through well-differentiated somites.

c—coelom; g—gut; mes—mesoderm; n—ventral nerve cord.

Coelom and Blood Vessels

After a short time a space appears in the centre of each somite, and it differentiates into an inner splanchnic layer and an outer somatic layer (Fig. 9.20). Since the space of the segments expand around the digestive tract from both sides towards the dorsal surface, the original small slit develops into a spacious coelom (Fig. 9.20). The splanchnic layer forms a flat tissue which gives rise to the peritoneum which surrounds the digestive tract. The somatic layer develops into cuboidal and columnar tissue, which mostly goes to form the muscular layer making up the body wall.

The cellular layers constituting the anterior and posterior walls of each segment unite with those adjacent to them to form septa between each two segments. The splanchnic layer of each of the paired somites expands to the dorsal and ventral sides of the digestive tract where the two layers meet and fuse, giving rise to a mesentery (Fig. 9.20). This kind of segmented mesoderm is not found in the prostomium. The first somite begins with the peristomium, but the septa between the segment and the mesenteries are likely to undergo secondary modifications in connection with such processes as the formation of the head and develop in various ways characteristic of the different species.

The blood vessels develop in the space left between the wall of the gut and the splanchnic layer of the somites. This space represents a remnant of the blastocoel, which has been compressed by the expansion of the coelom. Endothelial cells appear here and become organized locally into primitive vessels. These structures eventually become united end to end and below the digestive tract to form the dorsal and ventral blood vessels. In the same way lateral blood vessels, connecting the dorsal and ventral vessels also develop in each segment. These major vessels later divide into five branches to form a completely closed circulatory system.

In the Hirudinea also, the somites develop rapidly at first forming a large coelomic cavity in each segment, as in the Oligochaeta. By the time the characteristic leech sinus system, which corresponds to a circulatory system, is being formed, the coelomic cavities become filled with parenchyme cells, and secondarily retrogress. The left and right somites first unite above the ventral nerve cord, leaving a small space which becomes the ventral sinus. They then extend around the digestive tract to meet at its dorsal side, the narrow space left here forms the dorsal sinus. The septa between the segments disappear in the vicinity of the dorsal and ventral sinuses, but thicken laterally to form masses of mesoderm from which cells separate off and become mesenchyme, this tissue fills in the cavities of the coelom, at the same time giving rise to other spaces which form the lateral sinuses and botryoidal tissue.

Nephridia

In the Annelida a distinction is made between the larval and adult nephridia. The larval nephridium as is found in the trochophore stage of the protonephridial type. In the leech embryo the development of protonephridium is conspicuous. The embryo of *Nephelis* has two pairs of protonephridia; three pairs in *Hirudo* and four pairs in *Aulastoma*: In all the above cases they arise from a part of the ectodermal band. However, the adult nephridia are formed during the late embryonic period as segmental organs in each segment. A large number of experiments have been made in the oligochaete embryos, to elucidate the origin of the nephroblasts which produce these organs. The results of these observations are not agreed very well, with the result that there are at present two opposite views of the matter. One of these views says that the nephridia have an ectodermal origin, that at least the nephroblasts arise from the ectodermal cells comprising the ectodermal cell line m^1. (Wilson, 1899, *Lumbricus*, Vejdowsky, 1892, *Rhynchelmis*, Staff 1910, *Criodrilus*). The other view insists that the nephridia have a mesodermal origin. Bergh (1888, 1889) expressed this opinion with respect to *Criodrilus* and received support from many later workers (Panners 1923, *Tubifex*; Iwanoff, 1928, *Tubifex*, Vanderobock 1932, *Allolobophora*). The results of Lilli's (1905) observations on the polychaete, *Arenicola* also practically prove that the nephroblasts have a mesodermal origin. In the leeches, the appearance of the nephroblasts in the cellular layer forming the wall of the somites has been established. At any rate, although the nephroblasts show some special characteristics attributable to species difference, they always appear on the anterior part of the somatic layer in each of the regularly developed segments. Next they produce a small, winding line of cells which extends backwards between the somatic layer of the mesoderm and the ectoderm, this row of cells finally develops into a fine tubule which connects with the ectoderm to form a nephridium.

In the Echiuroidea, the protonephridia degenerate at metamorphosis; in their place there appears in the posterior part of the ventral side an anal sac which has three pairs of nephridial rudiments and will later form the genital ducts and nephridia (Fig. 9.19).

Reproductive System

The reproductive system develops at a later stage when all the vegetative organs have nearly completed their development. The formation of this system actually begins with the appearance of the primordial germ cells. These cells are separated from the line of body cells at a very early developmental stage, but fail to cleave further for some time thereafter, lying among the mesoderm cells of the splanchnic layer so that it is often difficult to trace their whereabouts. About the time the peritoneum reaches its full development, the primordial germ cells make their appearance in special regions of the septa for the first time becoming clearly evident as the gonadal rudiments. Since these cells have a characteristic vesicular nucleus, it is possible to distinguish them from the mesoderm cells.

In the polychaete *Solmacina* the first appearance of the primordial germ cells was detected in the gastrula stage by Malaguin (1925). The two mesodermal teloblasts (M^r and M^l) first separate off the primordial germ cells and then begin to produce the mesodermal bands. These paired cells lie in a bilaterally symmetrical position near the blastopore, but the rapid multiplication of the mesoderm cells derived from the teloblasts makes it difficult to distinguish them. However, they take no part in the mesodermal proliferation, dividing for the first time during the metatrochophore stage and dispersing into the somites, which are just developing at this time.

In the Oligochaeta the development of the primordial germ cells takes the same course. According to Penners and Stablieu (1930) on *Tubifex* and *Limnodrilus*, the primordial germ cells, which have separated from the mesodermal teloblasts, first undergo two divisions and then come to lie between the mesodermal bands and the endoderm cells (Fig. 9.21). As development proceeds and the segments are being formed one by one the primordial germ cells become buried in the septal tissue between the segments from the eighth to the twelfth (Fig. 9.21).

These primordial germ cells which are lodged in certain regions of the septa, form the gonads in these positions. In *Tubifex* the difference between the testis and ovary rudiments can first be seen about the time of the 27 segment stage (Meyer, 1929). In this stage the testis rudiment consists of one germ cell in the septum, while the ovarian rudiment is already made up of two germ cells (Fig. 9.21). As development progresses further, these cells continue to divide in situ, producing a number of germ cells, while at the same time the clearly different cells of the septum surround them to form a small lump of immature gonad (Fig. 9.21).

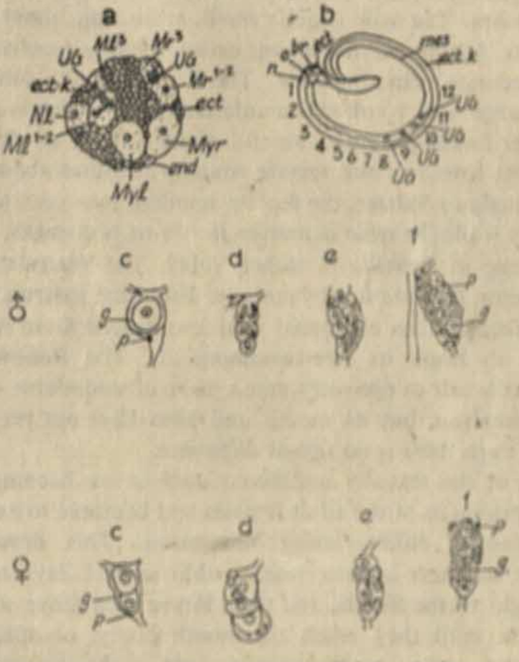


Fig. 9.21. Formation of gonad in *Tubifex rivolorum*.
a—Appearance of primordial germ cells at gastrula stage.
b—Orientation of primordial germ cells in late embryonic stage.
c-f—Process of gonad formation.
br—cerebral ganglion; ect—ectoderm; cet.k—ectodermal band; end—endoderm; g—germ cell; mes—mesoderm; ml.Mr, Myl, Myr—myoblasts; n—ventral nerve cord; Nl—left neuroblast; o—mouth; p—septal cell.

In Polychaeta the gonads are formed on a larger scale appearing in all the segments. As the breeding season approaches the germ cells undergo a rapid proliferation, in many cases being released into the body cavity and maturing there. The genital ducts and other accessory organs are formed last in the course of organogenesis, usually arising as modification of some part of the peritoneum. In some cases the nephridia also serve as genital ducts during the breeding season.

Sex Differentiation in Bonellia

The echiuroid *Bonellia* is famous for its exaggerated sexual dimorphism. The male is quite small, measuring about 1-3 mm in length. All the internal organs except the reproductive system are degenerate as in a parasite. The female on the other hand has a large oval trunk to the anterior end of which is attached a slender forked proboscis which is twice as long as the trunk. The total length of the female roughly measures about 15 cm. Accordingly to Baltzer, the female requires two years to reach maturity while the male is mature in one or two weeks.

The egg of *Bonellia* is rather yolky. The gastrulation and archenteron formation is by epiboly. From the gastrula stage it goes directly to an elongated oval metatrochophore stage and hatches to begin its free-swimming life. The free-swimming larva has a pair of eye-spots and a mass of endoderm cells for an archenteron, but its mouth and anus have not yet opened. At this stage there is no sexual difference.

Some of the sexually undifferentiated larvae become caught on the proboscis of the adult females and continue to exist there as symbionts, differentiating into males. This development towards maleness is determined within several days after they are caught by the female, and these larvae then move along the proboscis until they reach the mouth cavity, oesophagus, or some other region in the anterior end of the digestive tract. Here they develop exactly as though they were parasitic on female and reach maturity. Then they leave the digestive tract and invade the uterus. Several mature males are usually found in the uterus of a mature female and also a large number of developing males in the digestive tract. Those larvae which on the other hand undergo the latter part of development as free-living organisms, not connected with the adult female differentiate into females.

The eye-spots and ciliary bands of the male-determined larvae begin to degenerate but their external morphology and internal structure show evidence of their metamorphosis into males.

After about two days gonadal tissue containing spermatocytes accumulate in the anterior part of the mesodermal layer surrounding the body cavity, shortly afterwards mature spermatozoa can be seen in the body cavity. The mature males in the uterus of the female exhibit a fully mature organization, including a so-called seminal vesicle. This seminal vesicle is considered to have a unique embryonic origin, observable only in the male of *Bonellia.*

10. *Arthropoda*

1. ONYCHOPHORA

Among the modern arthropods, the Onychophora have long been recognized as a relict group whose morphology displays many primitive features. It is only to be expected, therefore, that the embryonic development of onychophorans should be highly pertinent to any comparison of annelid and arthropod embryology.

Most Onychophora have a highly specialized mode of development associated with a small, secondarily yolkless egg and viviparity. Only a few Australian species retain a yolky egg and of these, only one or two are oviparous, the remainder being ovoviviparous. The oviparous species *Symperipatus* and *Coperipatus* are also rare, have an astonishingly prolonged development (17 months) and are consequently unknown embryologically. The ovoviviparous species represent a primitive mode of development for Onychophora, in which the egg is long and proceeds to an advanced stage, with full complement of segments, at the expense of the yolk of the egg. The viviparous species fall into two groups, those of southern Africa, which develop at the expense of maternal nutrients but lack a placenta, and those of central and south America, have a placental attachment to the oviducal wall.

The yolky eggs of oviparous and ovoviviparous Onychophora are ovoid in shape, measure more than a millimetre and have a primitive centrolecithal structure. The egg cytoplasm is dense with yolk and devoid of periplasm and the zygote nucleus lies in a central position, surrounded by a small halo of yolk-free cytoplasm. Two membranes enclose the egg, a thin vitelline membrane and an external chorion which is thick in oviparous and thin in ovoviviparous individuals. The cleavage is intralecithal as found in many arthropods (Fig. 10.1.1) The yolk mass remains undivided while the zygote nucleus and the daughters divide and spread, with accompanying divisions of their cytoplasmic haloes. Some of the nuclei and their haloes rise to the surface of the yolk mass to form a small disc of blastomeres. With further division and spread, these cells then give rise to a low, cuboidal blastoderm at the surface. Simultaneously, the yolk mass divides into a number of yolk spheres. The cytoplasm of the egg breaks up into several spheres (Fig. 10.1.2) and each sphere is a nucleated blastomere, while the remainder are anucleate pseudoblastomeres. When mitosis begins, the blastomere divides into a disc of blastomeres apposed to the inner surface of the egg membranes on one side of the egg. The blastomeres absorb the disintegrating pseudoblastomeres. By the time 64 cells are present, the blastomere disc extends as a saddle around two-thirds of the circumference of the egg, leaving one side and both ends free of cells. Division continues (Fig. 10.1.2) and the saddle of blastomeres becomes a continuous blastoderm, which then swells by fluid uptake, stretching the enclosing membrane.

The placental viviparous Onychophora have even smaller eggs than those of the placental species and lack the recapitulatory swelling of the egg after release from the ovary. The eggs are 25-40 μ in diameter and the enclosing membranes are absent. Cleavage is total and equal, yielding a small morula which lies free in the lumen of the oviduct. With further division (Fig. 10.1.3) the morula becomes hollow, with a wall one cell thick, and attaches to the wall of the oviduct. The vesicle now enters directly into a further specialized phase of development, in which the cells of the attachment region proliferate as a hollow stalk, spread out basally as a flat placental plate against the oviducal wall while the remaining cells undergo further divisions which increase the size and cell number of the hollow vesicle at the free end of the stalk.

Presumptive Areas of the Blastoderm

Although the results of cleavage are highly variable among the onychophorans, the resulting pattern of presumptive areas of blastoderm (Fig. 10.1.4) is much more conservative. The

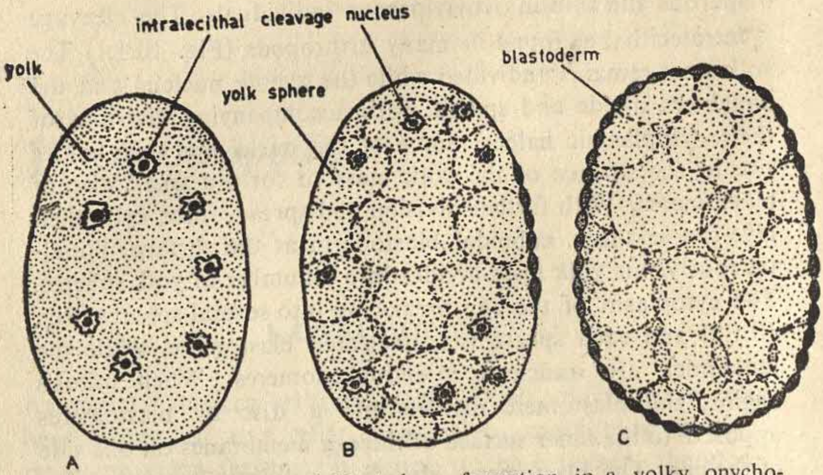


Fig. 10.1.1. Cleavage and blastoderm formation in a yolky onychophoran egg.
A—Intralecithal cleavage.
B—Initiation of blastoderm formation.
C—Blastoderm.

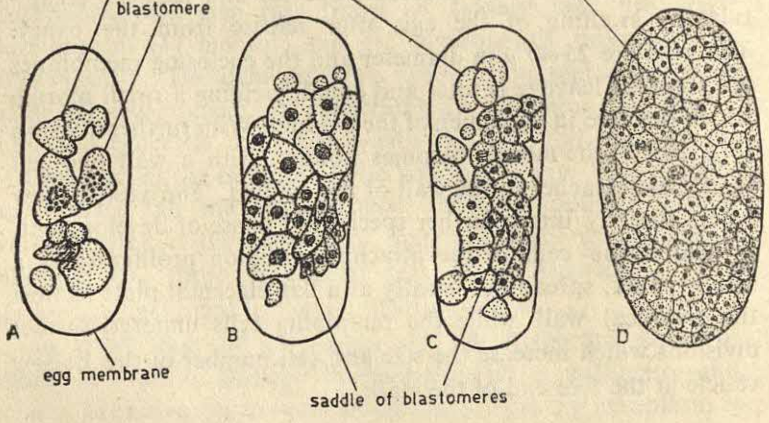


Fig. 10.1.2. Early cleavage in *Peripatopsis*.
A—2-cell stage.
B—Early saddle of blastomeres.
C—Later saddle of blastomeres.
D—Blastoderm before dilatation.

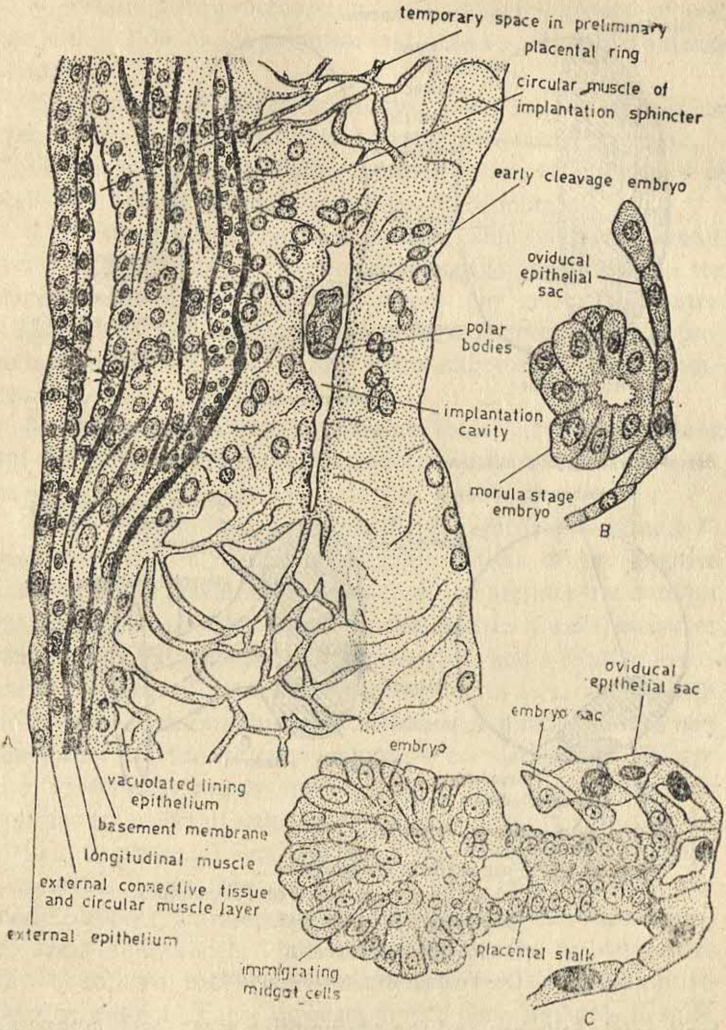


Fig. 10.1.3. Cleavage leading to formation of the stalked vesicle in a viviparous neotropical onychophoran.
A—Early cleavage embryo becoming implanted in oviduct.
B—Morula stage.
C—Stalked vesicle stage.

basic pattern for the group is displayed in species whose blastoderm covers a mass of yolk spheres. Anteroposterior and dorsoventral axes are fixed, the former corresponding to the

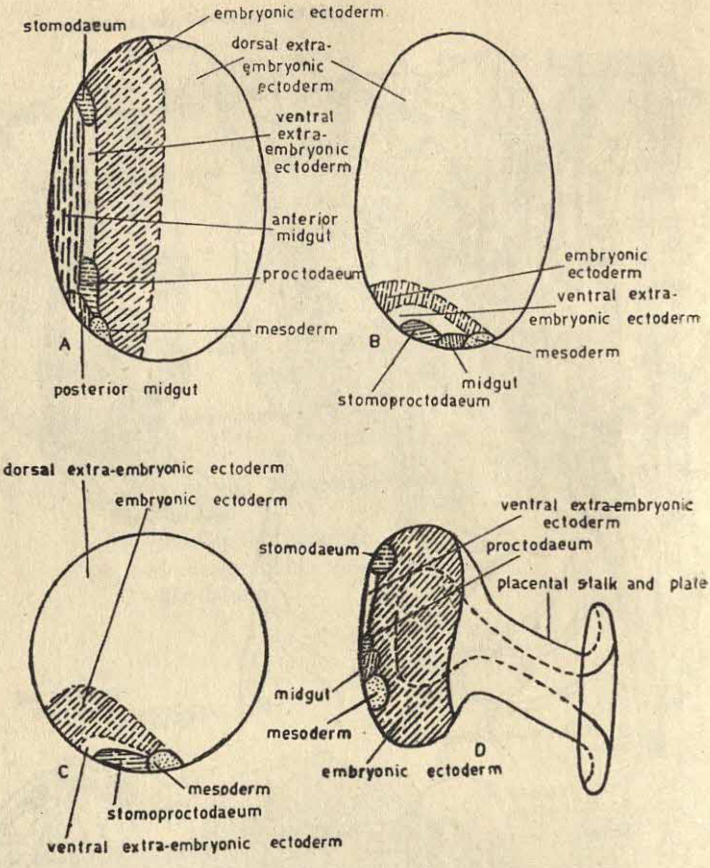


Fig. 10.1.4. Fate maps in Onychophora.
A—Yolky species based on *Peripatoides*.
B—*Peripatopsis sedgwicki*.
C—*Peripatopsis capensis*.
D—Viviparous placental species.

long axis of the egg and the presumptive areas, each composed of a sheet of small cells at the surface of the yolk as follows:

1) Presumptive anterior midgut, a long narrow band of mid-ventral cells.

2) Presumptive stomodaeum, an area of cells around the anterior end of the presumptive anterior midgut.

3) Ventral presumptive extra-embryonic ectoderm, a pair of narrow bands of cells flanking the presumptive anterior midgut.

4) Presumptive proctodaeum, a pair of small groups of cells on either side of the posterior end of the presumptive anterior midgut.

5) Presumptive posterior midgut, a small group of cells just behind the posterior end of the presumptive anterior midgut.

6) Presumptive mesoderm, a small posteroventral group of cells just behind the presumptive posterior midgut.

7) Presumptive embryonic ectoderm, extending as two broad, ventrolateral bands from the presumptive mesoderm to the anterior end of the blastoderm where they meet. The ventral edges of the presumptive ectoderm flank in succession, the presumptive proctodaeum, ventral extra-embryonic ectoderm and stomodaeum.

8) Presumptive dorsal extra-embryonic ectoderm making up the dorsal half of the blastoderm covering the large yolk mass.

In the viviparous species like *Peripatopsis moseleyi* and *P. sedgwicki*, the only differences in the pattern of presumptive areas of the blastoderm are absence of the presumptive anterior midgut, which in yolky species is the source of cells acting as temporary vitellophages digesting the yolk, and a relative increase in the area of extra-embryonic ectoderm as compared with embryonic ectoderm. The latter difference no doubt reflects a new functional role for the extra-embryonic ectoderm as an absorptive epithelium, convergently similar to the albumenotrophic yolk sac ectoderm of secondarily yolkless clitellate embryos. In viviparous placental Onychophora a consideration of presumptive areas shows that the placental stalk and plate area are modified dorsal extra-embryonic ectoderm and that the remaining areas are established in the usual way in the vesicular part of the embryo except for complete loss of the presumptive anterior midgut. Thus, throughout the Onychophora, in spite of great variations of the mode of cleavage, the presumptive areas formed as a result of cleavage show a remarkable stable configuration, only the presumptive midgut and the extra-embryonic ectoderm, the two areas intimately involved with yolk, being much affected by secondary loss of yolk.

Gastrulation in the Onychophora is different from any annelid, simply because the presumptive areas entering into gastrulation are a sheet of small, yolk-free cells at the surface of the yolk and include no large cells whatsoever. In the Onychophora, the extent to which the small-celled presumptive areas move as a whole is extremely limited. The presumptive areas enter into proliferative organogeny in their primary superficial positions, so that the cells which invade the interior of the embryo pass in as small cells through and around the yolk mass, and immediately begin to form organ rudiments. The presumptive anterior midgut in yolky onychophoran embryos, although a midventral sheet of small cells at the surface of the yolk, retains a definite and quite remarkable gastrulation movement (Fig. 10.1.5). The sheet of cells sinks inwards at the onset of gastrulation, then separates along the midventral line to leave the yolk broadly exposed along the ventral surface. The paired bands of sunken presumptive anterior midgut cells bordering the ventral slit now become mitotically active. Cells proliferated from these bands migrate upwards through the peripheral yolk, taking up yolk and acting as temporary vitellophages as they move. Eventually the temporary vitellophage cells form a complete yolky epithelium around the yolk mass, subsequently giving rise to the definitive epithelium of the anterior part of the midgut. At the same time, the presumptive posterior midgut sinks slightly inwards and begins to proliferate cells which are added to the midgut epithelium behind the level of the yolk mass. It is likely that posterior midgut proliferation is prolonged, extending the midgut along the growing trunk, but is completed before the full number of trunk segments is formed.

In the secondarily yolkless embryos of *Peripatopsis sedgwicki*, the presumptive anterior midgut and its temporary vitellophage function are lost. The entire midgut in these species is formed by cells proliferated from a slightly invaginated posterior midgut rudiment (Figs. 10.1.6 and 10.1.7). The cells spread forwards and upwards beneath the blastoderm to enclose a central, fluid filled space and continue to be proliferated until almost all of the trunk segments have been formed.

The midgut in the viviparous placental species arises by a prolonged proliferation from the presumptive posterior midgut, which sinks slightly inwards before proliferation begins (Fig. 10.1.8). The proliferated cells give rise to an epithelial sac. No trace of the presumptive anterior midgut is retained.

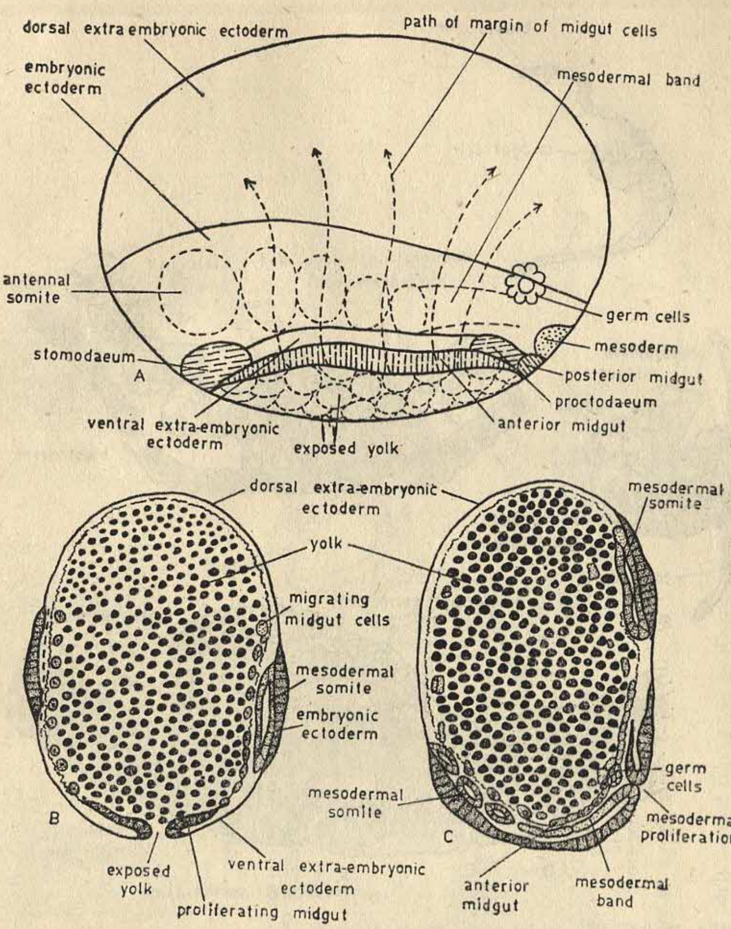


Fig. 10.1.5. Gastrulation and early segment formation in *Peripatoides*.
A—Diagrammatic lateral views.
B—Section showing immigrating midgut.
C—Section showing immigrating mesoderm and somite formation.

The same features as that of the midgut characterizes the penetration of the mesoderm into the interior in the Onychophora. In a constant manner, in all species, the presumptive mesoderm invaginates slightly and proliferates two streams of cells which migrate forward along paired, ventrolateral paths beneath the blastoderm (Figs. 10.1.5, 10.1.6, 10.1.7 and 10.1.8).

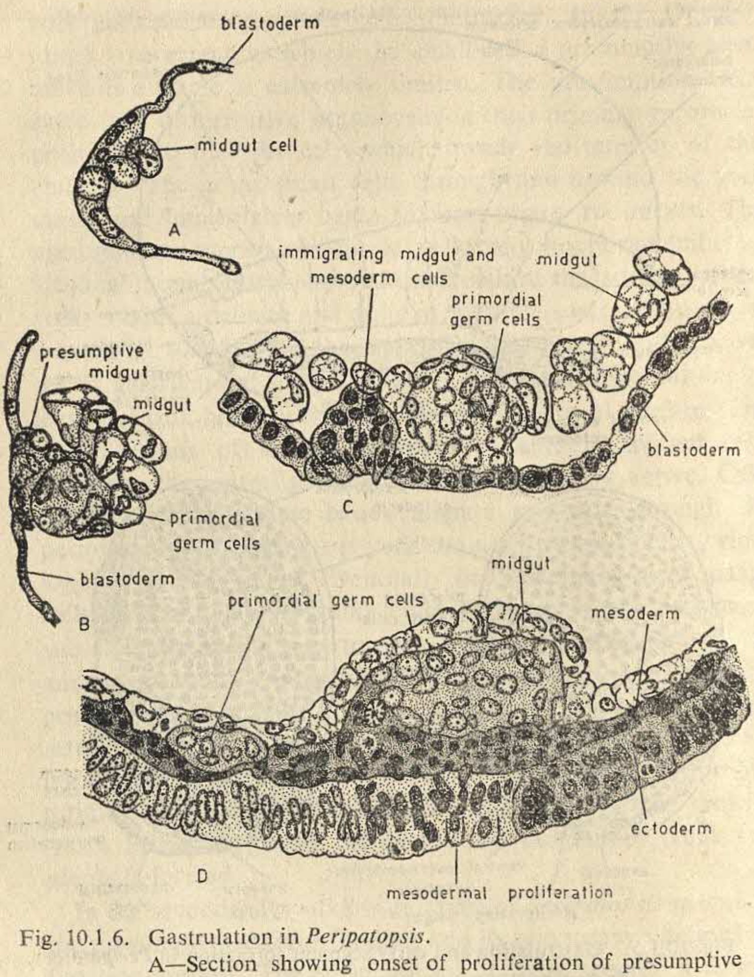


Fig. 10.1.6. Gastrulation in *Peripatopsis*.
A—Section showing onset of proliferation of presumptive midgut.
B—Sagittal section showing later midgut proliferation.
C—Sagittal section at onset of proliferation on presumptive mesoderm.
D—T.S. showing later mesoderm proliferation.

Proliferation continues till the mesoderm of all segments has been formed, by which time, the anterior part of the mesodermal bands is well advanced into organogeny.

Gastrulation activity of the presumptive stomodaeum and proctodaeum is more complex, being closely linked with that

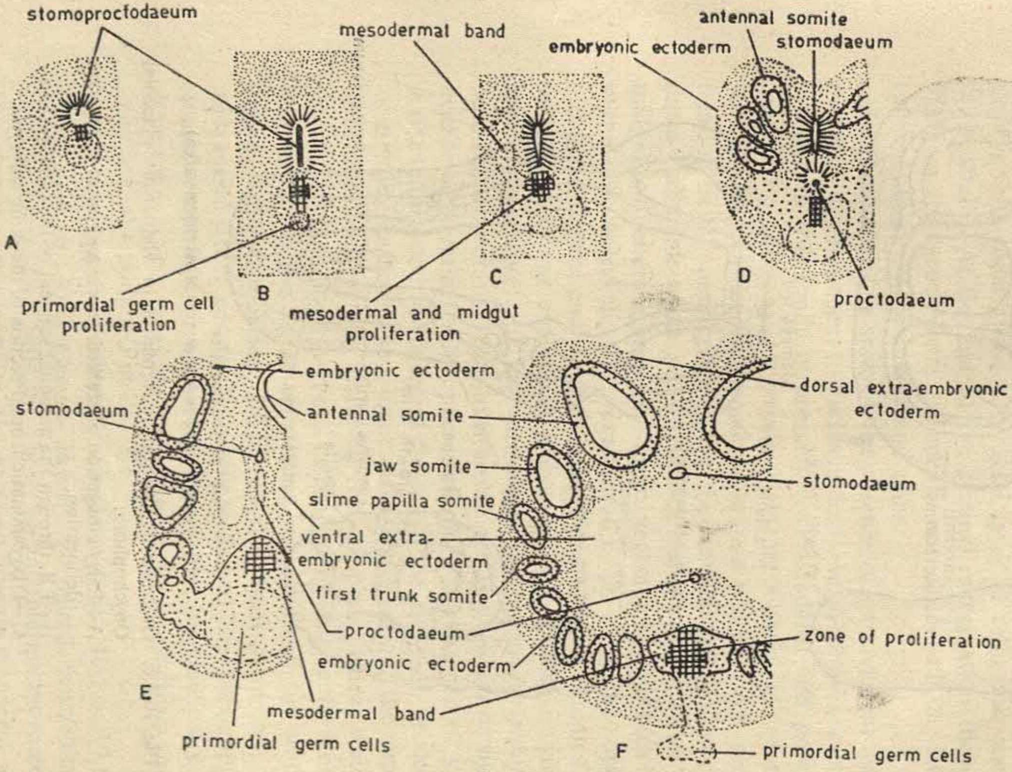


Fig. 10.1.7. Gastrulation and early somite formation in *Peripatopsis*.

A—Presumptive areas.
C—Early mesoderm proliferation.
B—Formation of stomoproctodaeum.
D—Separation of mouth and anus, early somite formation.

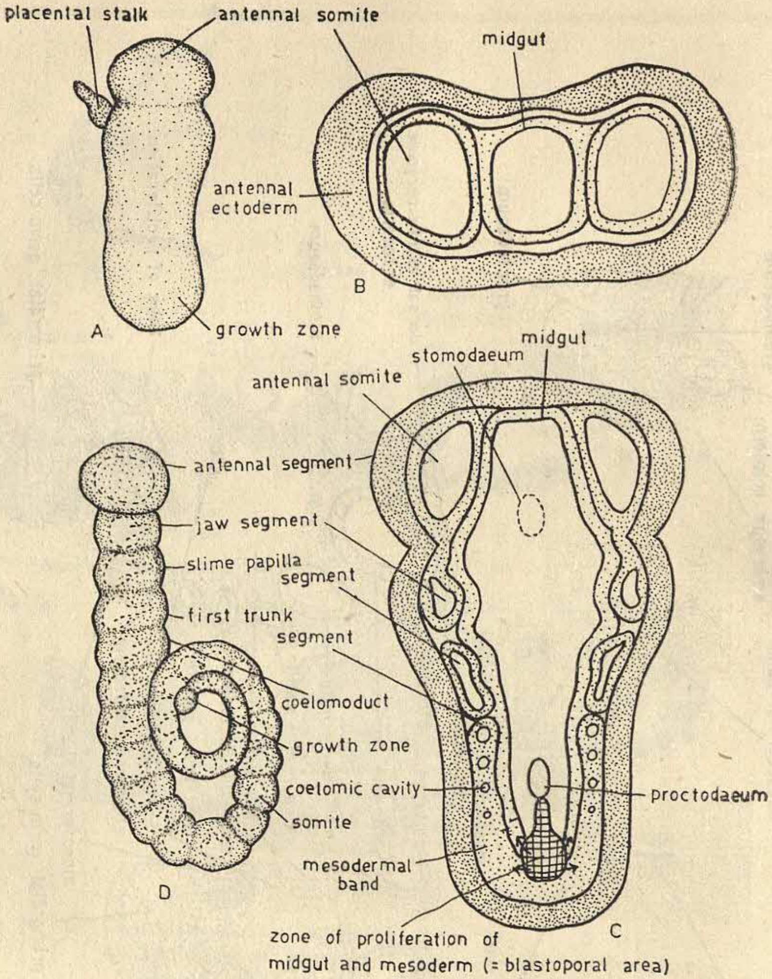


Fig. 10.1.8. Gastrulation and somite formation in viviparous placental Onychophora.

A—Early elongation of stalked vesicle and onset of segment delineation.

B—T.S. through the antennal segment of the same embryo.

C—Diagrammatic frontal section of the same embryo.

D—Coiled segmenting embryo in lateral view.

of the presumptive anterior midgut. In yolky embryos (Fig. 10.1.5) the stomodaeal and proctodaeal areas invaginate at the ends of the midventral, presumptive midgut invagination.

When the midgut bands are fully internal, the lips of the invagination merge together along the ventral midline, except at the ends. Internally, the paired midgut bands meet midventrally. Externally as a result of gastrulation spread of the ventral extra-embryonic ectoderm, the superficial layer of cells also meets midventrally. At the end of the original invagination the stomodaeal and proctodaeal rudiments are closed off as short tubes, opening by the mouth and anus.

In *Peripatopsis*, in spite of the absence of a midventral anterior midgut component in the blastoderm, much of this sequence is retained. The confluent presumptive stomodaeal and proctodaeal areas invaginate together producing a short midventral slit (Fig 10.17). The slit then elongates as a result of cell proliferation in its lateral lips, pushing the stomodaeal and proctodaeal rudiments apart at the ends of the slit. By this time the midgut epithelium is present as a sac, ventral wall of which lies within the ventral slit. The ventral wall of the midgut sac opens along the ventral midline and the margins of the opening fuse with those of the superficial midventral slit. Just as in yolky species, therefore, the interior of the midgut in *Peripatopsis* becomes temporarily open to the exterior. The functional significance of retention of this phenomenon in the absence of yolk is not understood. Midventral closure of the slit now proceeds as in yolky species. The midgut cells separate from the superficial cells and reunite, restoring the complete ventral wall of the midgut sac. The superficial cells also unite along the ventral midline, at the same time completing tubulation of the stomodaeum and proctodaeum. In placental viviparous species, the midventral slit is completely eliminated, and the presumptive stomodaeum and proctodaeum invaginate independently as short tubes which break through secondarily to establish continuity with the midgut (Fig 10.1.8.)

The presumptive embryonic ectoderm (Figs. 10.1.5, 10.1.6, 10.1.7 and 10.1.8) is formed directly as paired ventrolateral bands of blastoderm cells and shows no gastrulation movement. The dorsal component of the extra-embryonic ectoderm also lacks this activity, being formed initially as a broad area of blastodermal cells covering the yolk. In contrast, the paired ventrolateral bands of extra-embryonic ectoderm in the yolky and the viviparous (non-placental) come together to the ventral midline by a gastrulation spreading movement which completes the ectodermal covering of the embryo. In the placental species the same component is narrow and unpaired and forms directly in its definitive position, a secondary simplification.

Consideration of the gastrulation movements in the onychophoran embryos bears out the fact, that when the yolk is absent, it is absent secondarily. The movements which occur are obviously basically related to the sitting out of a blastoderm around a large, internal yolk mass. Two further generalizations at this point are: (1) when a blastoderm is formed, gastrulation movements are largely eliminated in favour of precocious proliferation, especially of the presumptive mesoderm and midgut; (2) presumptive areas of the same location, relative juxtaposition and fat may be formed as: large cells containing yolk; small cells at the surface of yolk; a small group of cells which proliferates and spreads during gastrulation; or a broad area of cells formed directly as a result of cleavage. The second point is of special importance in comparing the development of blastula-forming and blastoderm forming species.

Further Development of the Gut

In all species the stomodaeum and proctodaeum develop directly, through cell division and differentation as the foregut and hindgut epithelia. Further development of the midgut is more diverse. In yolky embryos, in which the anterior part of the midgut is filled with yolk and has a wall of temporary vitellophage function, further development of the midgut proceeds partly by resorption of yolk and partly by the addition of further cells from the posterior midgut rudiment. Both the temporary vitellophage portion and the posterior portion then differentiate directly after further cell divisions, as midgut epithelium. Viviparous, non-placental species show various modifications of this condition. In yolky species, the yolk-filled midgut is mainly covered by the ectoderm. As elongation of the embryo proceeds, the midgut cells enter into mitosis and become more compactly arranged once more, accompanied by shrinkage of the anterodorsal sac, to form the definitive midgut epithelium. The viviparous placental Onychophora also show a direct and simplified development of the midgut epithelium proliferated from the presumptive area of posterior midgut (Fig.

10.1.8). No specialization of the midgut is associated in these species with the modification of the dorsal extra-embryonic ectoderm as a placenta.

Development of External Form

All onycophorans are epimorphic, attaining the full complement of segments and adult organ systems before they hatch or are born as juveniles. In spite of the frequency of yolklessness and viviparity among the species, the development of the external form of the embryo is not greatly variable.

Development always proceeds in strict anteroposterior succession. As the mouth and anus are being formed, the paired lateral halves of the embryo begin to develop as thickened bands on the surface of the blastoderm and soon exhibit paired segmental annuli. The first pair of swellings develops as the two halves of the antennal segments, in a preoral position in front of the mouth. The two halves of the antennal segment are always in contact in the anterior midline. Behind them, on either side of the mouth, the two halves of the jaw segment arise followed by the slime-papilla segment and a series of trunk segments. Trunk segment formation continues at the posterior end at a relatively rapid rate, so that limb buds do not begin to appear on the anterior segments until most of the trunk segments have been formed. Due to acceleration of segment formation relative to further development of the segments, the full complement of segments is formed before the anterior segments have progressed far along their pathway of further development.

In the yolky embryos of *P. novae-zealandiae*, the segment halves of the anterior part of the embryo are widely separated on either side of the yolk mass (Fig. 10.1.9). As segment proliferation continues, the posterior part of the embryo is prolonged beyond the yolk mass as a tubular caudal papilla, in which the two halves of each segment are only slightly separated by narrow mid-dorsal and mid-ventral prolongations of the extra-embryonic ectoderm. Associated with the development in a confined space enclosed by ovoid membranes, the elongating caudal papilla is fixed forwards ventrally beneath the yolk filled anterior part of the embryo. As the development of limb buds continues and the full number of trunk segments is gradually attained, the yolk within the anterior part of the embryo dimi-

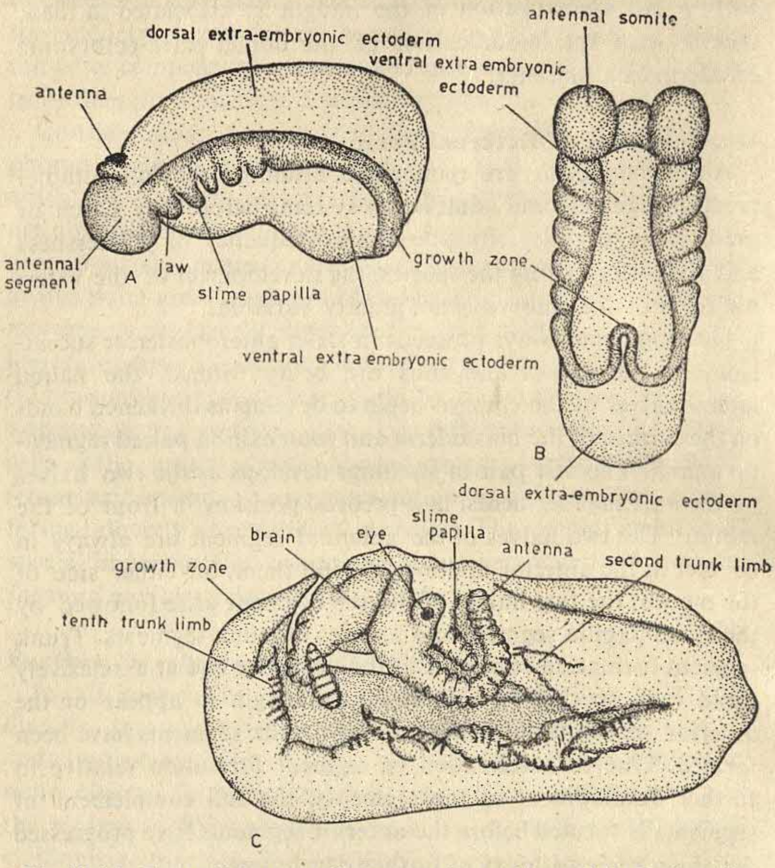


Fig. 10.1.9. External features of segmentation and growth in *Peripatoides*.
A—Early segmented delineation, lateral view.
B—The same stage in ventral view.
C—Flexed embryo, lateral view.

nishes in volume. This part, therefore, also becomes tubular, with two halves of each segment coming together in the midline, first ventrally, later dorsally. The final flexed position of the embryo within the egg membrane results from this change.

In those species of *Peripatopsis* which retain a swollen sac of dorsal extra-embryonic ectoderm and still develop within enclosing egg membranes (Figs. 10.1.10 and 10.1.11), this sequence of development is modified only in minor ways. The ventral separation of the two halves of the anterior segments is slight, but

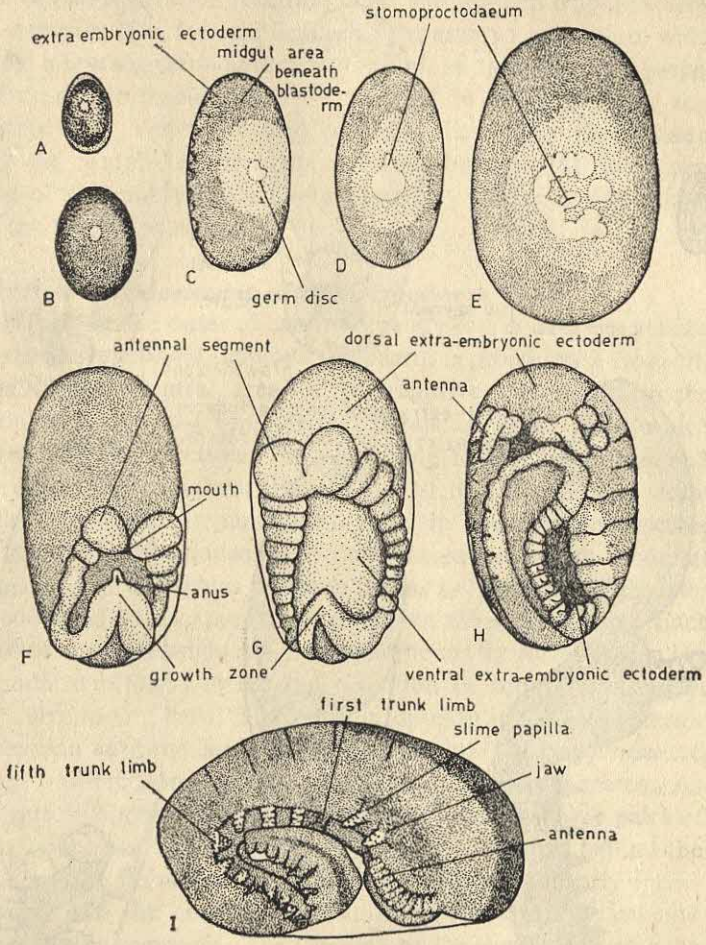


Fig. 10.1.10. External features of segmentation and growth of *Peripatopsis*.
A—Blastoderm before dilatation.
B & C—Early dilatation and formation of the embryonic primordium.
D—Further enlargement of the embryonic primordium.
E—Initial segment delineation.
F & G—Continued segment formation and ventral flexure.
H—Fully segmented embryo.
I—Older flexed embryo after resorption of dorsal extra-embryonic ectoderm.

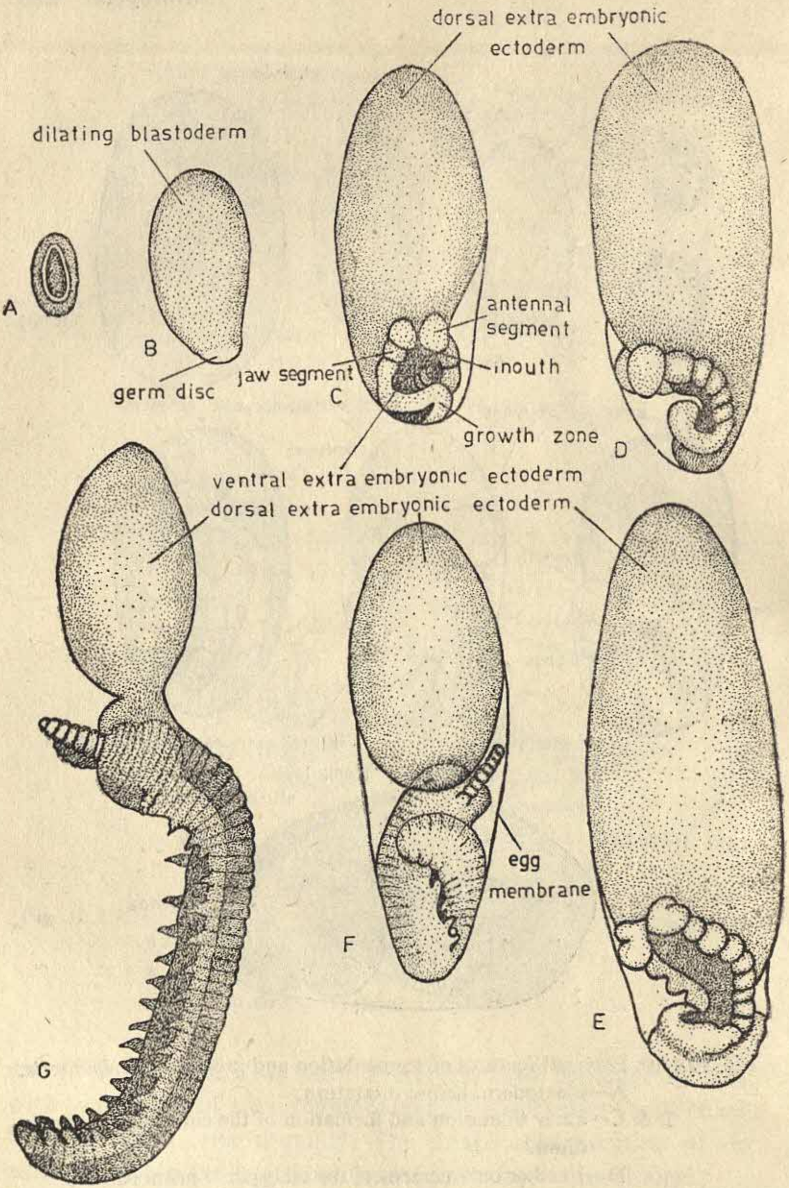


Fig. 10.1.11. External features of segmentation and growth of *Peripatopsis sedgwicki*.

A—Blastoderm before dilatation.

B—Dilated blastoderm with embryonic primordium.

C, D & E—Stages in segment formation and ventral flexure.

F—Older embryo with shrinking trophi c vesicle.

G—Slightly older embryo, unfolded to show details of head and trunk.

the dorsal separation resulting from the swollen trophic vesicle is extensive. The forward flexure of the embryo takes place when only a few segments have been formed, so that the dorsal separation due to trophic vesicle is confined to a few anterior segments only. Ventral formed flexure of the embryo persists during the completion of segment formation, and the increasing size of the embryo is accommodated by progressive shrinkage of the trophic vesicle.

Further Development of the Mesoderm

Preceding the onset of external manifestation of segmentation in the Onychophora, copious mesoderm is proliferated from the small posteroventral area of presumptive mesoderm, in the form of two broad ventrolateral bands of small cells which spread forwards between the developing midgut epithelium and the overlying blastoderm (Figs. 10.1.5 to 10.1.8). The mesoderm cells push forwards until they meet in the anterior midline in front of the stomodaeum. Somite formation, which is similar in yolky and viviparous embryos begins as the ends of the two mesodermal bands approach the level of the stomodaeum. Each pair of somites originates by coelomic cavity formation in the mesoderm followed by the individuation of somite walls from the surrounding cells. The somites develop in antero-posterior succession until the last segmental pair has been individuated when proliferation from the presumptive mesoderm ceases. As a result of continued forward migration, the first three pairs of somites come to lie in front of, precisely at, and just behind the levels of the stomodaeum. These pairs are subsequently incorporated into the head as the somites of the antennal, jaw and slime papilla segments. The swelling of the somites initiates the superficial delineation of the segments throughout the head and trunk. The initial change following swelling of a somite is partial subdivision into three compartments, dorsolateral, medioventral and appendicular. Only the appendicular compartment contributes cells which differentiate as somatic musculature. As this compartment pushes outwards, the overlying ectoderm protrudes as a segmental limb bud. The wall of the somite in this vicinity then disaggregates and spreads as a mesenchyme which differentiates into intrinsic and extrinsic limb musculature, circular muscle and longitudinal muscle of its half segment (Figs 10.1.12 and 10.1.13).

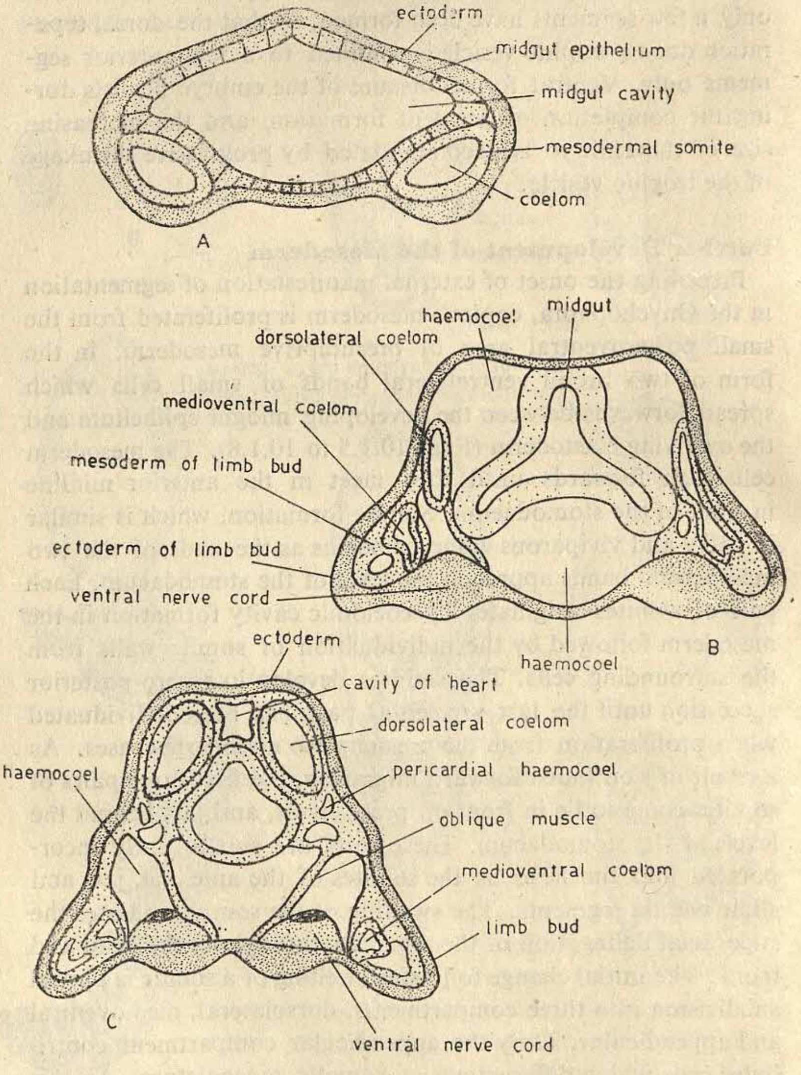


Fig. 10.1.12. Diagrammatic transverse section showing the early stages of segment differentiation in *Peripatopsis capensis*.

With the transformation of somite walls partially into mesenchyme, the coelomic cavities become confluent with spaces which develop between the mesenchyme cells. An extensive

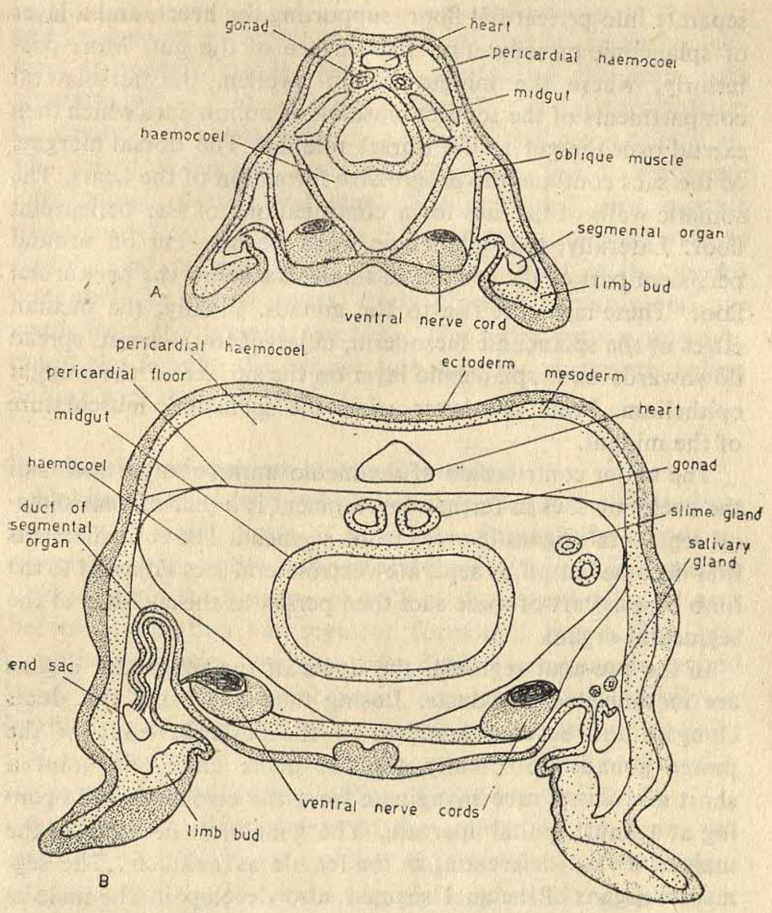


Fig. 10.1.13. Diagrammatic transverse sections showing the later stages of segment differentiation in *Peripatopsis capensis*.

system of blood spaces thereby results, forming the haemocoelic body cavity of the developing animal. The only definite vessel is the mid-dorsal heart, formed by cells of the upper margins of the dorsolateral compartments of the somites. Anteriorly, if the somites are widely separated, the walls of these compartments initially become mesenchymatous and push up towards the dorsal midline over the surface of the swollen anterior midgut. The marginal cells then differentiate as heart walls around a middorsal haemocoelic space, while the more lateral cells separate into pericardial floor, supporting the heart, and a layer of splanchnic mesoderm on the surface of the gut. More posteriorly, where the midgut is not swollen, the dorsolateral compartments of the somites close off as hollow sacs which then extend over the gut to the dorsal midline. The dorsal margins of the sacs continue the progressive formation of the heart. The somatic walls of the sacs form continuations of the pericardial floor. Laterally, the splanchnic walls become cut off around persistent part of the coelom as small sacs below the pericardial floor. These later give rise to the gonads. Finally, the median edges of the splanchnic mesoderm, adjacent to the heart, spread downwards as a splanchnic layer on the surface of the midgut epithelium. From this layer arises the splanchnic musculature of the midgut.

The major contribution of the medioventral compartments of the coelomic sacs in further development is a pair of ventrolateral segmental organs in every trunk segment. The compartments first become cut off as separate ventrolateral sacs adjacent to the limb bases. Part of these sacs then persist as the end sacs of the segmental organs.

In the pre-anal segment, the ducts of the segmental organs are modified as gonoducts. Losing their end sacs, the ducts elongate and become attached to the posterior ends of the paired gonads. Proximally, the two ducts unite and joint a short midventral tube invaginate from the ectoderm and opening at a single genital aperture. The gonoducts develops in the male as a vasa deferentia, in the female as oviducts. The segmental organs of the anal segment also develop in the male as accessory genital structures. In females these organs are lost.

The primordial germ cells are segrégated during early gastrulation, as a group of cells which migrates into the interior with the early mesoderm or posterior midgut cells and congregates beneath the proliferating presumptive mesoderm (Figs. 10.1.5 to 10.1.7). The same genital rudiment is formed whether yolk is present in the embryo or not. From this position, the germ cells migrate to the dorsal edges of the somites. Many of the primordial germ cells degenerate before reaching somites. The region of the somite wall in which the germ cells lie is the splanchnic wall of the dorsolateral compartment of each somite. As the lateral part of this wall merges into the pericardial floor and the median part spreads over the surface of the gut as incipient splanchnic muscle, the part containing the germ cells separates off as a closed vesicle beneath the pericardial floor, the cavity of the vesicle being a pinched off piece of coelom. The paired vesicles of successive segments fuse to form two longitudinal, dorsolateral tubes. The germ cells now migrate into the coelomic lumen of the tube and begin to proliferate as oogonia and spermatogonia. The posterior ends of the gonads unite with the paired genital ducts growing forwards and upwards from the pre-anal segment. The testes remain separate, but in the female, the ovaries join together posteriorly before uniting with the genital ducts.

Further Development of the Ectoderm

Unlike the mesoderm, which is represented in the blastoderm by only a small group of cells, the presumptive ectoderm, especially in the yolky embryos and those which retain a swollen blastoderm in the absence of yolk is already extensive in area before gastrulation and segment formation begins. The presumptive ectoderm comprise three distinct components, paired bands of presumptive embryonic ectoderm ventrolaterally, a broad area of presumptive extra-embryonic ectoderm dorsally and a paired band of extra-embryonic ectoderm, becoming a single united band, ventrally (Figs. 10.1.4 and 10.1.5)

In the further development of the embryonic ectoderm, the majority of the cells of the presumptive bands of embryonic ectoderm give rise to ectoderm of the head. Most of the trunk ectoderm is proliferated from cells at the posterior ends of these bands, which form an ectodermal growth zone on either side of the presumptive mesoderm, budding off cells in a forward direction. In the small embryos of viviparous placental species and of non-placental species lacking a swollen yolk sac, it seems likely that the ectoderm of all the trunk segments is formed in this way.

As the paired bands of mesoderm push forwards beneath the blastoderm, the blastoderm cells outside them begin to divide and become cuboidal (Fig. 10.1.5). At the same time, adjacent blastoderm cells begin to concentrate into the same region, so that the two bands of embryonic ectoderm become dense, while the temporary yolk sacs ectoderm above and below them becomes attenuated. Formation and swelling of somites also precedes the division of the embryonic ectoderm into segmental units outside the somites (Figs. 10.1.5 to 10.1.8). The intersegmental grooves that mark these units traverse only the embryonic ectoderm, without extending on to the extra-embryonic ectoderm. The ectodermal segmental units do not become distinct until the anterior somites have completed their forward migration on either side of the stomodaeum, so that the head ectoderm develops from blastoderm cells that are already in the appropriate position.

When the embryo is large, whether yolky or non-yolky, the posterior parts of the bands of presumptive embryonic ectoderm give rise directly to the ectoderm of several trunk segments and each half segment is widely separated from its partner by broad dorsal and ventral areas of extra-embryonic ectoderm. More posteriorly in the segments proliferated from the growth zone the two halves of each segment are separated by only narrow strips of extra embryonic ectoderm. The extra embryonic ectoderm is later mostly transferred into definitive segmental ectoderm. Before discussing this, let us examine the further development of the embryonic ectoderm outside each pair of trunk somites (Figs. 10.1.12 and 10.1.13). The products of this layer are the surface epithelium, ventral nerve cord, trachea, coxal glands and exit ducts of the segmental organs. The trachea and coxal glands do not develop until after birth or hatching. The ventral nerve cords arise in association with so-called ventral organs. At an early stage in the development of each trunk segment, the ventrolateral embryonic ectoderm of each side of the segment begins to bud off small cells into the interior. A thickening is formed which soon divides into two parts. The lateral part, continuous from segment to segment, sinks into the interior to give rise to the ventral nerve cord of its side. The median part becomes segmentally distinct and then partially invaginates, to form a so-called ventral organs on each side of the segment. As the ventral epithelium of each segment is completed, the ventral organs fuse in the ventral midline, then gradually shrink and disappear. Their function is still unknown. The remainder of the embryonic ectoderm gives rise to the surface epithelium of the segments, pouching out ventrolaterally as the surface epithelium of the limb buds. As the segment halves spread around the gut, the extra-embryonic ectoderm is for the most part transformed into surface epithelium along the dorsal and ventral midlines.

Composition of the Onychophoran Head

With the onychophoran head, we enter into the different question of the incorporation into the pre-oral part of the head of structures which appear to be formed initially as post-oral segments. Whether these components of the head are segments or not has been the subject of argument and counter-argument. As we have already seen three pairs of somites lie in front of the first trunk segment. The first pair are large and lie anterior to the stomodaeum. The second and third pairs, somewhat smaller, lie on either side of the stomodaeum and behind the stomodaeum respectively. These three pairs of somites (Fig. 10.1.7) originate posteriorly and migrate forwards to comprise the entire mesoderm of the head. External to the three pairs of head somites, the anterior parts of the bands of presumptive embryonic ectoderm become marked off as the ectoderm of three segments, a large pre-oral antennal segment, a jaw segment flanking the mouth and a slime-papilla segment behind the mouth (Fig 10.1.14). The two haloes of the antennal segment already meet in the midline when first formed, but the jaw segment and slime-papilla segment are divided dorsally and ventrally by extra-embryonic ectoderm, just like the trunk segments which follow them.

The course of further development confirms that the jaw segment and slime papilla segment are undoubtedly cephalized segments belonging to the same metameric series as the trunk segments. The somites become subdivided into the usual three compartments of which the lateral, appendicular compartments grow out with the ectoderm as limb buds, the jaws and slime papillae respectively. The paired medioventral compartments in the slime-papilla segment develop coelomoducts, which then becomes greatly elongated, retaining their coelomic end sacs to form salivary glands. The proximal ends of the two glands later come together in the ventral midline to open by a common pore into the pre-oral cavity. The jaw segment lacks coelomoducts. Its small somites are mainly concerned in the formation of specialized jaws muscles. In fact, the major products of two

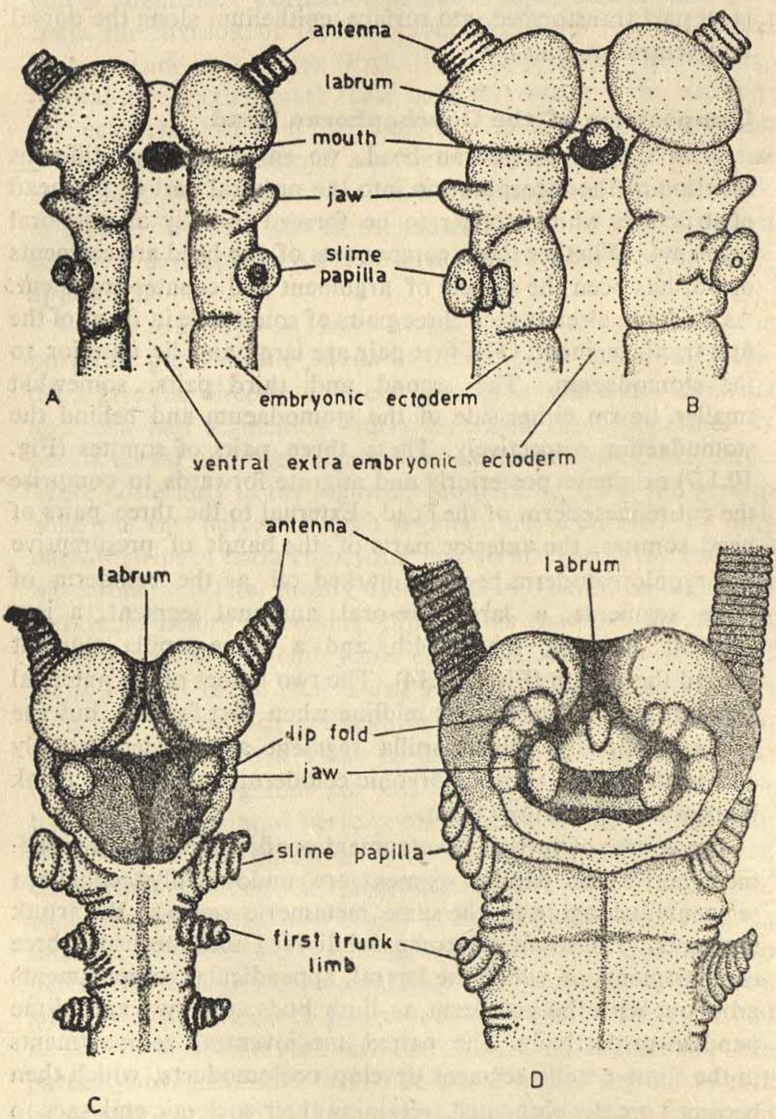


Fig. 10.1.14. Development of the Onychophoran head in external view.
A and B—Early stage in formation of the head of *Peripatopsis capensis.*
C and D—Later stage in formation of the head of *Epiperipatus trinidadensis.*

pairs of somites are the muscles of the foregut, jaws and slime-papillae, but the details of their development including the fate of the dorsolateral components, require further study. These somites do not contribute to the heart.

The ectoderm of the jaw segment and slime-papilla segment also follows essentially the same course of development as that of the trunk segments. Surface epithelium and ventral nerve cords result, the latter being associated with paired ventral organs that are later resorbed. The ventral organs of the jaw segment lie just behind the posterior lip of the mouth. The only ectodermal specializations observed in these segments is the invagination of the ectoderm of the tips of the slime-papillae as long slime glands growing back into the trunk. Whether the pre-oral part of the onychophoran head constitutes more than a single cephalized segment that has migrated forward in front of the mouth is a more controversial question. The paired antennal somites are obviously serially homologous with those behind them, becoming subdivided into three compartments, developing a pair of limb buds in association with the appendicular compartments and forming a pair of transient coelomoducts ventrally. Beyond this they develop as antennal musculature, foregut muscles and other muscles of the head. The pre-oral ectoderm also shares many features in common with the post-oral segmental ectoderm. Much of it develops as surface epithelium of the head and antennae. Over the broad ventralateral area on each side the ectoderm thicken as antennal ganglionic rudiments, continuous with the ventral cords, from which arise the paired ganglia of the brain and the circumpharyngeal connectives. Part of each thickening also remains at the surface and develop as a temporary ventral organ.

It can be concluded that the major part of the pre-oral region of the head in Onychophora comprises a cephalized segment that has moved forwards in front of the mouth as the antennal segment. All the necessary segmental criteria of somites, limbs, coelomoducts, paired ganglia and associated ventral organs are fulfilled. The only question that cannot be answered at the present time is whether the pre-oral part of the head in Onychophora retains any pre-segmental components. Whether the brain incorporates anterior components, perhaps intimately associated in origin and structure with the antennal ganglia, that might be equated with a pair of protocerebral ganglia, has yet to be decided. That a pre-segmental component of the brain was present in the ancestors of Onychophorans seems highly probable, since it is retained as the entire brain in annelids and is identifiable as large protocerebral ganglia in all myriapods.

Just as a pre-segmental acron cannot be identified in Onychophora, so also there is no external sign of a post-segmental pygidium or telson. In this case, however, the absence of such a structure finds a reasonable explanation in terms of the total mode of development. The growth zone from which the trunk segments arise is post-anal in position, having ectoderm on either side of posteroventral mesoderm, when the last mesoderm is proliferated, the growth zone ectoderm develops directly into the ectoderm of the anal segment and no post-segmental ectoderm remains.

The Basic Pattern of Development on Onychophora

The basic pattern of development in the Onychophora proceeds from a large yolky egg, deposited by an oviparous female and protected by two membranes, an outer chorion and an inner vitelline membrane. Development is epimorphic, all segments of the body being developed before hatching takes place, but involves the anteroposterior addition of several trunk segments beyond the length of the original egg, formed by proliferation from a terminal growth zone. The increasing length is mainly added while the yolk mass within the embryo is still voluminous, and is accommodated within the limited egg space by forward flexure of the growing region beneath the ventral surface of the anterior region.

The egg undergoes intralecithal cleavage, forming a blastoderm around a mass of yolk spheres. The blastoderm has a characteristic distribution of presumptive areas, each composed of small cells at the surface of the yolk. The presumptive midgut, a long midventral band of cells, is in two parts, a long anterior midgut and a compact posterior midgut behind it. The presumptive anterior midgut splits along the ventral midline exposing the yolk, and invaginates slightly as paired cell bands. These now proliferate cells, which migrate around the yolk, taking up part of the yolk material, and form a diffuse vitellophage epithelium at the surface of the yolk mass. The ventral split subsequently closes so that the vitellophage epithelium becomes continuous. After completion of its vitellophage function this layer of cells transforms into the definitive epithelium of the anterior part of the midgut. The posterior midgut rudiment remains superficial and proliferates cells which form a tubular continuation of the midgut epithelium in the growing trunk, behind the yolk-filled anterior region.

The presumptive stomodaeum and proctodaeum lie at the ends of the presumptive anterior midgut. The former is a short arc of cells, the latter a paired rudiment, when the invagination of the anterior midgut takes place, the stomadaeal and proctodaeal rudiments form the anterior and posterior margins of the ventral opening. As the lateral margins of this opening subsequently come together in the ventral midline, the stomodaeum and proctodaeum are closed off as short tubes, which subsequently gain openings into the midgut. The presumptive posterior midgut lies behind the anus at the end of this process.

Behind the presumptive posterior midgut is a second group of cells, posteroventral in position, the presumptive mesoderm. The cells of this area sink slightly below the surface and proliferate two streams of mesoderm cells, which pass forwards as paired lateral bands on either side of the midgut and eventually meet in the anterior midline. During forward growth, the mesodermal bands begin to segment into paired somites. Each somite is formed by individuation of a layer of mesoderm cells around a coelomic space and undergoes considerable swelling, developing a large coelomic cavity. Forward penetration of the mesodermal bands carries the first and largest pair of somites into a pre-oral position. The second pair comes to lie on either side of the mouth, the third pair just behind the mouth. These are the three paired somites of the head; antennal, jaw and slime papilla respectively. The remaining pairs are the somites of the trunk. The somites are formed in anteroposterior succession. The last mesodermal cells produced by the presumptive mesoderm enter into the last pair of somites and there is no residual mesoderm when hatching takes place.

The trunk somites become partially subdivided into three compartments, dorsolateral, medioventral and appendicular. The appendicular compartment pushes out as a lobe and then becomes mesenchymatous, giving rise to the somatic musculature (circular, dorsal longitudinal, ventral longitudinal, oblique, extrinsic limb and intrinsic limb muscle). The dorsolateral compartment extends towards the dorsal midline. Its somatic wall gives rise to the heart and pericardial floor. The splanchnic wall merges into the pericardial floor, and medially below the heart, gives rise to splanchnic mesoderm spreading downwards around the midgut. The medioventral compartment develops a coelomoduct and becomes the coelomic end sac of a segmental organ, subsequently lost in the first three segments. The coelom is otherwise obliterated or gains continuity with the haemocoel, except where it persists within the gonads. Primordial germ cells are segregated as early products of the proliferating presumptive mesoderm. The germ cells initially form a compact group of cells in a posteroventral position just below the surface, but then migrate outwards and upwards to enter the splanchnic walls of the more posterior trunk somites. Subsequently these splanchnic areas close off a small portion of the dorsolateral coelom on each side of the body as the cavity of a tubular gonad, and the germ cells migrate into the gonad cavities. The gonoducts are specialized coelomoducts of the preanal segment.

The somites of the head develop in a slightly modified manner but are serially homologous with the trunk somites. Antennal coelomoducts are only transient and the jaw segment lacks coelomoduct vestiges, but those of the slime papilla segment become specialized as salivary glands.

The presumptive ectoderm comprises all except the ventral part of the bastoderm. External to the mesodermal bands, it concentrates as dense bands of embryonic ectoderm. Dorsally and ventrally, it becomes attenuated as extra-embryonic ectoderm, later incorporated into the segmental ectoderm. The ventral component of the extra-embryonic ectoderm is paired at first and borders the ventral presumptive areas (stomodaeum, anterior midgut, proctodaeum and posterior midgut) but with the closure of the ventral opening, the two halves come together as a midventral band.

Segmental subdivision of the embryonic ectoderm follows swelling of the paired somites in the anteroposterior succession. The intersegmental grooves do not extend to the extra-embryonic ectoderm. Each segmental unit of ectoderm ventrally thickens with the lateral part of the thickening forming a ventral nerve cord, the median part a temporary ventral organ. The remainder of the ectoderm gives rise to surface epithelium, including ventrolateral outpouchings as limb buds, and lateral invaginations as coxal glands and tracheae. The surface epithelium spreads dorsally and ventrally and incorporates the extra-embryonic ectoderm.

The posterior ends of the paired bands of presumptive embryonic ectoderm, lying on either side of the presumptive mesoderm, constitute a growth zone from which the ectoderm of the more posterior trunk segments is proliferated. The greater zone ectoderm finally forms part of the ectoderm of the anal segment and there is no post-segmental pygidium. Since the growth zone lies behind the anus, which is formed before proliferation of new segments begins, the ectoderm and mesoderm of new segments must pass forwards on either side of the anus as the trunk grows larger.

The ectoderm of the jaw segment and slime papilla segment develops in essentially the same way as that of the trunk segments. The slime glands are probably modified coxal glands. As far as can be seen, the pre-oral ectoderm external to the antennal somites also develops in the same manner, though its ganglionic components are disproportionately enlarged. Whether the preoral region of the head includes any presegmental components of ectoderm, anterior to the cephalized antennal segment, cannot be answered satisfactorily on present evidences.

Superficially the basic pattern of embryonic development in Onychophora appears to have almost nothing in common with that of polychaete annelids beyond paired somites and metameric segmentation. Again, superficially, it appears to be quite different from that of clitellate annelids, i.e., annelids which show a particular response of basic annelid development to increased yolk and protective environment for development.

2. MYRIAPODA

All myriapod eggs exhibit the same structural organization. The egg is ovoid or spherical with a central nucleus in a small halo of yolk-free cytoplasm, surrounded by a dense accumulation of yolk granules enclosed in a sparse reticulum of cytoplasm. A single membrane, usually termed the chorion, encloses the egg. The eggs show a lot of variation in their sizes. The ovoid eggs of *Scolopendra cingulata* is 3 mm long, while that of *Scutigera coleoptrata* has a diameter of about 1–2 mm. Diplopod eggs are generally smaller, ranging from 1.3 mm in *Strongylosoma guerinii*, through 0.96 mm in *Platyrrhacus amauros*.

Cleavage

In spite of the differences in size, the cleavage always leads to the formation of the blastoderm. In *Scolopendra cingulata*, repeated nuclear divisions take places within the yolk mass and most of the resulting cleavage energids migrate to the surface of the yolk mass and emerge in small groups of cells uniformly scattered over the surface (Fig. 10.2.1). With further divisions and spreading, groups of cells merge to form a uniform low blastoderm. The remaining energids persists within the yolk mass as vitellophages. Another view of the cleavage in *Scolo-*

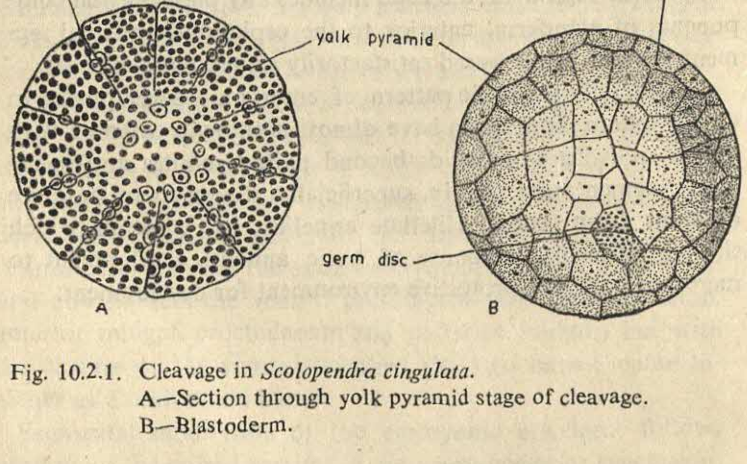


Fig. 10.2.1. Cleavage in *Scolopendra cingulata*.
A—Section through yolk pyramid stage of cleavage.
B—Blastoderm.

pendra is of the yolk pyramid formation. According to this the yolk mass divides into a number of radial yolk pyramids during early cleavage, converging on an undivided central mass of yolk. It is inferred that the yolk pyramids are anucleate and that the cleavage energids which migrate to the yolk surface do so by passing along the interfaces between pyramids. A more precise description of *Scolependra* cleavage is needed, but on the present evidence, cleavage can be regarded as essentially of the intralecithal type. As the blastoderm is completed, the yolk pyramids fuse once more to form a unitary nucleated yolk mass.

In the groups, diplopods, symphylans and pauropods, total cleavage precedes blastoderm formation (Figs. 10.2.2, 10.2.3, 10.2.4). After each nuclear division, a superficial furrowing initiates the interposed cleavage plane, but the deeper parts of the plane are formed as a thin sheet of cytoplasm which segregates within the yolk mass and then splits between the two cells. In consequence of this mode of sub-division, the spherical outline of the egg is maintained throughout cleavage. The first division divides the egg into two equal blastomeres. The second which is slightly less equal, is perpendicular to the first. The equal divisions continue, but become more irregular in timing pyramidal yolky blastomere, begin to be formed around a small, central blastocoel. Each pyramidal blastomere contains a nucleus and associated cytoplasmic halo (energid) and at each division, the energids move closer to the exposed outer surfaces of the pyramid. At approximately the 16-cell stage, some of the nuclei begin to undergo radial mitosis, cutting off polygonal yolky cells towards the centre of the egg and leaving truncated pyramids peripherally. The latter also continue to divide with tangential mitosis, when about 130 cells have been formed, the cell boundaries begin to disappear and as cleavage continue a unitary yolk mass is restored with numerous energids at its periphery and others more centrally placed. Finally the peripheral energids are cut off uniformly at the surface of the yolk mass as small, cuboidal blastoderm cells. Finally it appears as a uniform low blastoderm around a nucleated yolk mass.

In the Symphala (Figs. 10.2.2 and 10.2.3) radial mitosis do not begin until about 100 pyramidal blastomeres have been formed. A superficial layer of small, cuboidal cells, still containing some yolk, is then cut off from an inner mass of large, polygonal

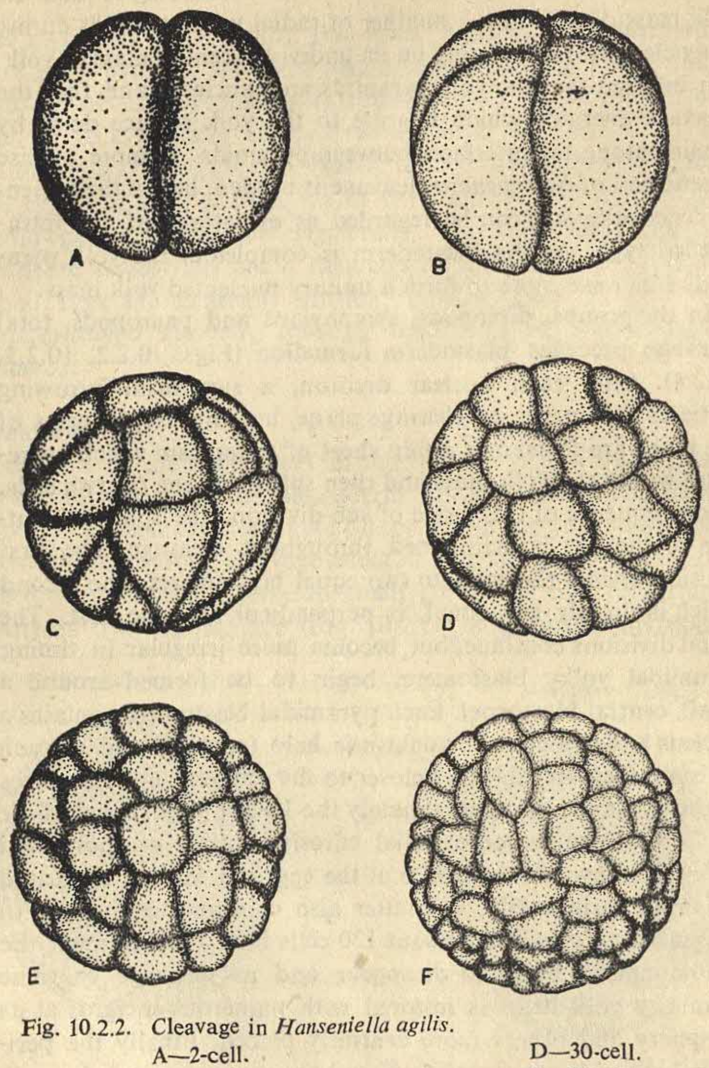


Fig. 10.2.2. Cleavage in *Hanseniella agilis*.
A—2-cell.
B—4-cell.
C—17-cell.
D—30-cell.
E—50-cell.
F—100-cell.

yolky cells surrounding the small blastocoel. The peripheral cells resume tangential divisions but they retain their cell boundaries and give rise directly to a columnar blastoderm. The divisions proceed in such a way that the cells of the ventral half

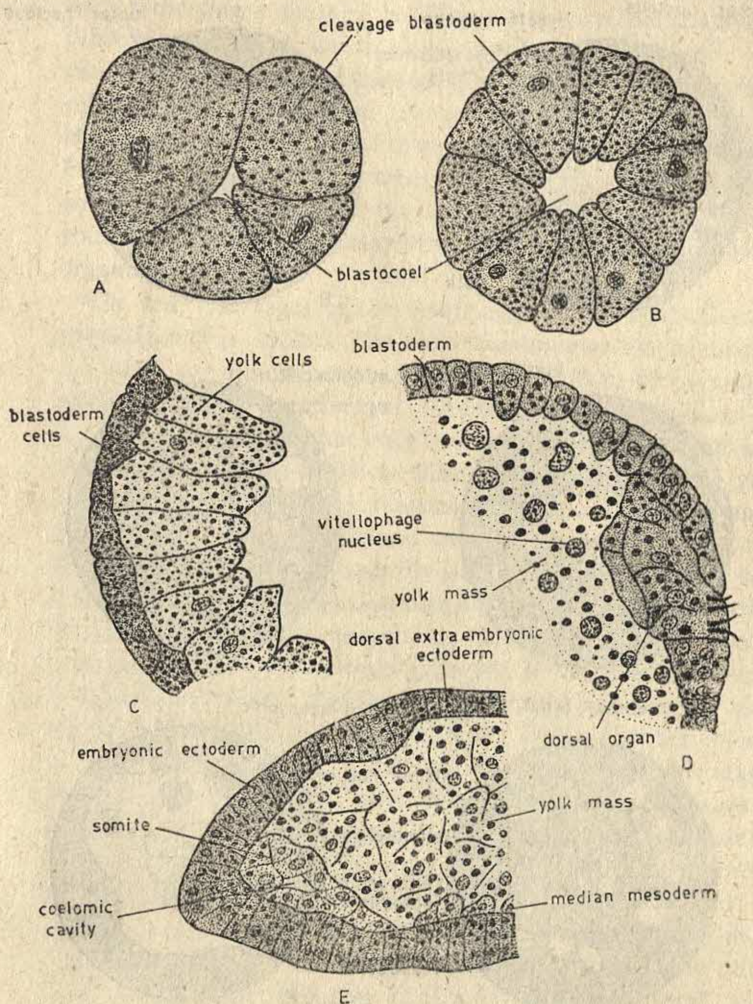


Fig. 10.2.3. *Hanseniella agilis*.

A—T.S. through 4 cell stage.

B—Section through an egg with 58 blastomeres.

C—Section through an egg with more than 120 cells.

D—Section through completed blastoderm with dorsal organ.

E—T.S. through 4th trunk segment at the stage of somite formation.

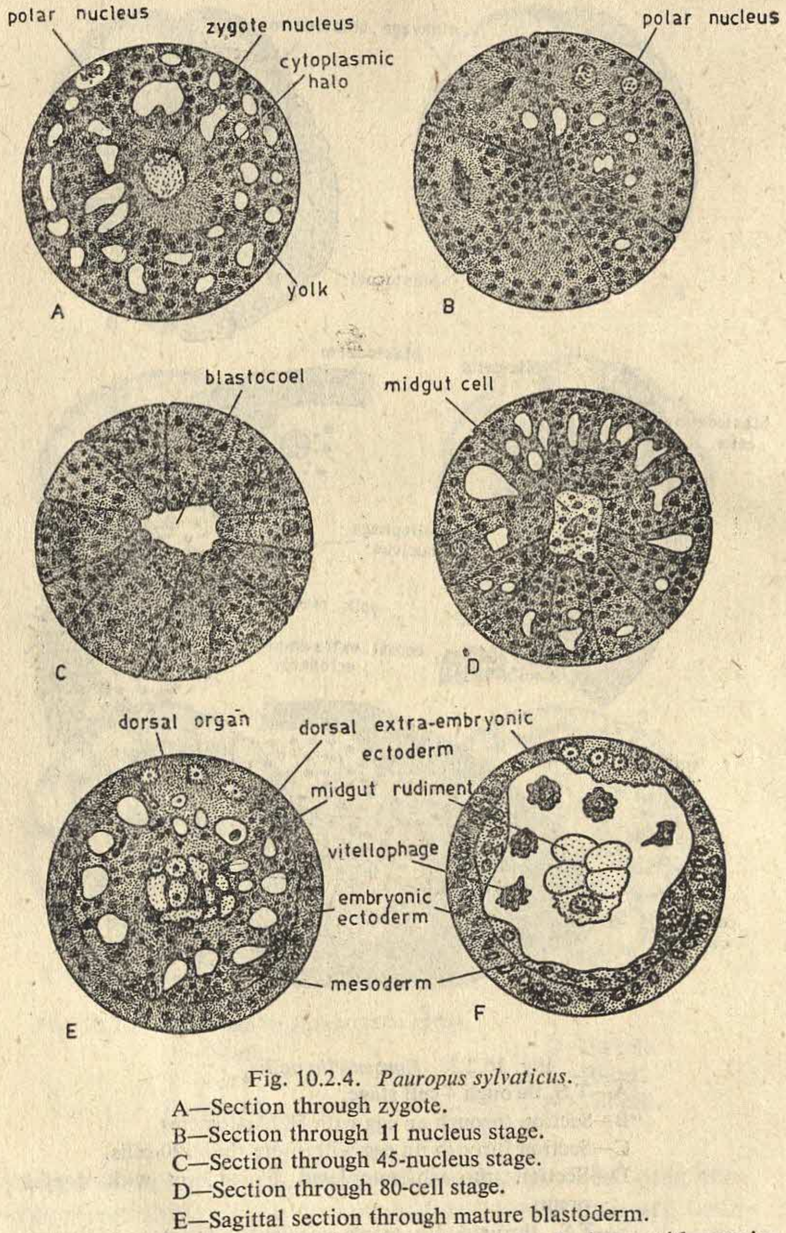


Fig. 10.2.4. *Pauropus sylvaticus.*

A—Section through zygote.

B—Section through 11 nucleus stage.

C—Section through 45-nucleus stage.

D—Section through 80-cell stage.

E—Sagittal section through mature blastoderm.

F—Sagittal section through germ hand, shown with anterior end on the right.

of the blastoderm are about twice as deep as those of the dorsal half, indicating a precocious differentiation of the embryonic primordium and a dorsal, extra-embryonic ectoderm. The actual cells, meanwhile undergo further radial mitosis, cutting off roughly a double layer of small irregular cells beneath the blastoderm, from a central group of anucleate yolk masses. Finally, as blastoderm formation is completed, the yolk masses and the irregular cells around them fuse to form a nucleated yolk mass within the blastoderm, establishing a blastodermal organization.

The later cleavages of the small eggs of *Pauropus* are more specialized (Fig. 10.2.4). The early blastomeres are small and the nuclei are almost peripheral by the time 40 to 45 pyramidal blastomeres have been formed. Generalized radial divisions do not occur at this stage but only two yolky cells are cut off into the centre of the egg to fill the blastocoel. Meanwhile, the yolk pyramids continue their tangential mitotic divisions until 160—200 cells are present and each pyramid is more or less columnar. During this phase, divisions are more numerous ventrally than dorsally, indicating a highly precocious differentiation of the embryonic primordium and the dorsal extra-embryonic ectoderm. Additional cytoplasm begins to accumulate around the peripheral nuclei, leaving only the central parts of the cells yolky. Further divisions continue wholly with tangential spindle so that no cells are cut off into the interior at this stage. Instead, the yolky inner parts of the cells separate inwards as anucleate yolk masses beneath a superficial layer of blastoderm cells and fuse together to form an anucleate yolk shell between the blastoderm and the two central yolky cells. As a result of this the blastoderm encloses not a nucleated mass, but an anucleate shell of yolk around two central, yolky cells. The outer yolk subsequently becomes nucleated and the central yolk becomes different from the peripheral yolk.

When complete, the blastoderm of diplopods, Symphala and Pauropoda secrete a thin but highly resistant blastodermal cuticle beneath the chorion. The chilopod blastoderm does not secrete a blastodermal cuticle.

Presumptive Areas of the Blastoderm

The construction of fate maps for myriapod blastoderm is more difficult than for any other group of arthropods. The difficulty arises partly because many myriapod embryos already have cells internal to the blastoderm at the blastoderm stage and is also complicated by the fact that the presumptive rudiments segregate from one another in a diffuse manner during subsequent development.

The presumptive midgut

The midgut epithelial cells of *Scolopendra* arise as cells which separate inwards in scattered array throughout the ventral surface of the blastoderm, leaving a continuous blastodermal epithelium at the surface. The midgut cells spread around the yolk mass to form an enclosing midgut epithelium, but do not enter the yolk mass and vitellophages. In the Symphala the yolk nucleus originate as a result of radial divisions during cleavage, but only the more laterally placed nuclei become the nuclei of fat-body cells. Those lying along the central cone of the yolk mass become the nuclei of midgut cells. Presumptive midgut in the Symphala is thus segregated precociously in the form of yolk cells during cleavage and retains no representation in the blastoderm.

The midgut rudiment of diplopods is a strand of cells which runs through the centre of the yolk mass. It was shown that the strand is proliferated from a localized postero-ventral area of the blastoderm, which must therefore be deemed the presumptive midgut. This area lies immediately in front of the presumptive proctodaeum. The yolk mass itself is occupied by nuclei resulting from radial mitosis during early cleavage. These act as vitellophages and then develop, as the yolk is gradually resorbed into fat body.

The Pauropoda show an extreme specialization, in which the presumptive midgut is cut off during early cleavage as the two distinct yolk cells in the centre of the yolk mass and the more peripheral yolk is populated at a later stage by vitellophages released by radial divisions throughout the early blastoderm. The vitellophages later develop into fat-body cells, as in the diplopods and Symphala.

When one takes into account that the chilopod fat body is a product of the mesoderm, it is clear that the chilopods and the remaining myriapods exhibit two alternative conditions of the presumptive midgut and the fat-body. In the chilopods, the midgut originates through a broadly spread, radial immigration of ventral blastoderm cells which migrate around the yolk mass. Vitellophages arise as persistent cleavage energids and as invasive cells proliferated from a small postero-ventral area of blastoderm in the midline behind the presumptive proctodaeum. Fat body arises from mesoderm. In the remaining myriapods, the presumptive midgut either originates by proliferation of a strand of cells through the centre of the yolk from a small postero-ventral area of blastoderm in front of the proctodaeum (diplopods) or is cut off in this internal position during cleavage (Symphala and Pauropoda). Vitellophages arise throughout the forming blastoderm as a result of radial divisions and later develop as fat body (Fig. 10.2.5).

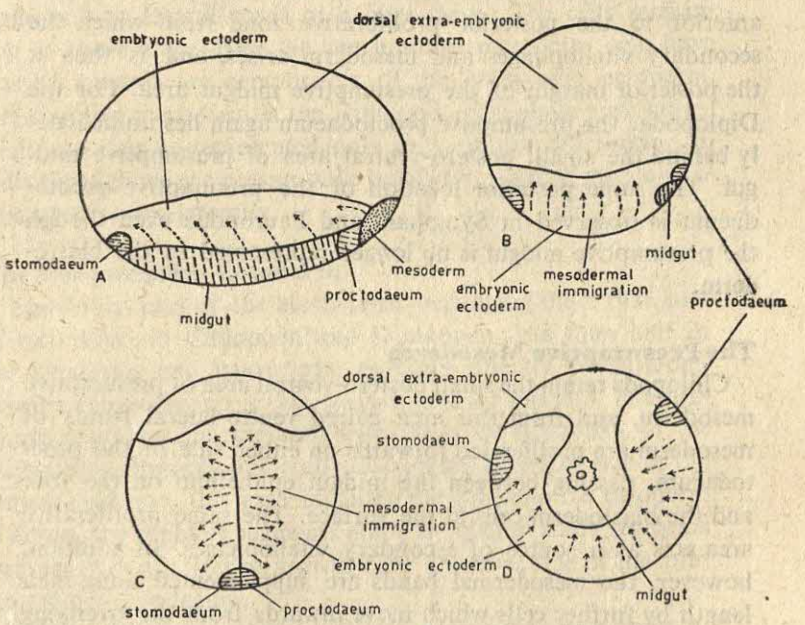


Fig. 10.2.5. Fate maps of the myriapod blastoderm.
A—Chilopoda.
B—Diplopoda.
C—Symphala.
D—Pauropoda.

The Presumptive Stomodaeum and Proctodaeum

The stomodaeum and the proctodaeum retain a uniformly simple development throughout the myriapods. Each arises as a cellular ingrowth in the ventral midline, the stomodaeum anteriorly between the incipient procephalic lobes, the proctodaeum posteriorly. The ingrowth hollows out as a short blind-ending tube. Localization of the presumptive stomodaeum of chilopods indicates a likely occupation of the anterior margin of the area from which midgut cells arise. For the Diplopoda, Symphala and the Pauropoda, the presumptive stomodaeum obviously cannot be identified in the same way, since there is no long ventral component of presumptive midgut in the blastoderm, but it can be placed quite easily relative to presumptive embryonic ectoderm.

The presumptive proctodaeum in the Chilopoda lies just anterior to the posterior proliferative zone from which the secondary vitellophages and mesoderm arises, and is thus at the posterior margin of the presumptive midgut area. For the Diplopoda, the presumptive proctodaeum again lies immediately behind the small, postero-ventral area of presumptive midgut. The same posterior location of the presumptive proctodaeum is observed in Symphala and Pauropoda even though the presumptive midgut is no longer represented in the blastoderm.

The Presumptive Mesoderm

Chilopods retain the small postero-ventral area of presumptive mesoderm, and from this area paired ventro-lateral bands of mesoderm are proliferated forwards on either side of the proctodaeum, passing between the midgut epithelium on the yolk and the blastoderm cells at the surface. The same proliferative area acts as a source of secondary vitellophages. In addition, however, the mesodermal bands are supplemented along their length by further cells which move inwards from the overlying blastoderm. Other cells migrate inwards from the ventral blastoderm between the two mesodermal bands, to lie beneath the surface as scattered median mesoderm. Thus, the presumptive mesoderm of chilopods has, in part a localized distribution postero-ventrally in the blastoderm, but in part is also diffusely spread bilaterally along the presumptive embryonic ectoderm.

The embryos of the Diplopoda, Symphala and Pauropoda show this diffuse pattern in a more modified form. The localized postero-ventral area of presumptive mesoderm is absent. Mesoderm cells separate into the interior diffusely over the entire length of the embryonic primordium. In the Symphala and probably also in diplopods the majority of mesoderm cells pass in along the lateral margins of the embryonic primordium and pile up as paired lateral cords. Immigration nearer the midline also produces a thin, irregular sheet of median mesoderm between the two lateral cords. In Pauropoda it is slightly modified, in that the cells separate into the interior over the entire surface of the embryonic primordium to form an irregular layer beneath the columnar outer layer and only begin to aggregate as lateral cords at a later stage. The cells remaining at the surface after the presumptive mesoderm cells have passed inwards are components of the embryonic ectoderm. Presumptive mesoderm in the Diplopoda, Symphala and Pauropoda can thus be represented only as a diffuse distribution of cells throughout the presumptive ectoderm, normally more concentrated ventro-laterally.

The Presumptive Ectoderm

The dorsal part of the blastoderm, occupying more than half of the surface in Chilopoda and Diplopoda, less than half in the Symphala and Pauropoda, develops directly as extra-embryonic ectoderm and is thus presumptive for this fate. The presumptive embryonic ectoderm arises from the remainder of the blastoderm. Broadly bilaterally distributed on either side of the ventral mid-line, this area includes the presumptive ectoderm of acron, the cephalic segments and a number of trunk segments together with a posterior growth zone on either side of the presumptive proctodaeum.

Before attaining its strict condition as presumptive ectoderm, the blastodermal area which has this subsequent fate releases many cells into the interior. As we have seen in chilopods, presumptive midgut cells are released first throughout the area, and presumptive mesoderm cells later, mainly along paired bilateral zones. In the diplopods and Symphala, there is again a mainly bilateral release of mesoderm cells and in the small embryo of *Pauropus*, a generalized release of mesoderm cells.

Gastrulation

Associated with the diffused nature of the presumptive areas of myriapod embryos and with modifications of cleavage in the small eggs of symphalans and pauropods, gastrulation is scarcely represented as a phase in myriapod development.

The presumptive midgut displays a gastrulation movement in the Chilopoda, as a diffuse migration of cells into the interior over a wide area. The diplopod presumptive midgut enters directly into organogeny in its superficial position. The presumptive midgut of Symphala and Pauropoda is cut off precociously into the interior during cleavage and exhibits the gastrulation movement.

The presumptive stomodaeum and proctodaeum of all myriapods undergo independent invagination as short tubes which then enter directly into organogeny. The posterior area of presumptive mesoderm in chilopods shows a slight insinking but then proceeds directly into proliferative organogeny without moving into the interior as a whole. The bilateral supplementary mesoderm of the chilopods enters the interior by diffuse migration, as does the entire presumptive mesoderm of the remaining myriapods.

The presumptive embryonic ectoderm and the dorsal extra-embryonic ectoderm enter directly into organogeny in the positions in which they are formed as components of the blastoderm. The presumptive fat body, in the form of vitellophages, is cut off into the interior during cleavage in diplopods, symphalans and pauropods. The chilopod fat body has a later origin from mesoderm.

Myriapods, then, have all but abandoned gastrulation as a significant phase in development and attain the arrival of the cells in their organ forming positions through a variety of specialized segregations during and immediately following blastoderm formation.

Further Development of the Gut

In contrast to the complex manner in which myriapods segregate and re-distribute their presumptive embryonic rudiments during early development, the further development of each rudiment during organogeny is simple. Simplest of all is the further development of the gut. The stomodaeal and proctodaeal rudiments grow into the interior as blind ending tubes in the usual way and re-differentiate as the epithelia of the foregut and hindgut respectively. The free end of the proctodaeum also pouches out as a pair of evaginations which form the Malpighian tubules. The stomatogastric ganglia originate as dorsal thickenings of the wall of the stomodaeum.

The midgut of *Scolopendra* (Fig. 10.2.6) develops directly from the epithelial layer of small midgut cells formed around the yolk mass. The vitellophages within the yolk mass play no part in midgut formation and are digested with the yolk. In diplopods, in contrast, the midgut epithelium develops from the

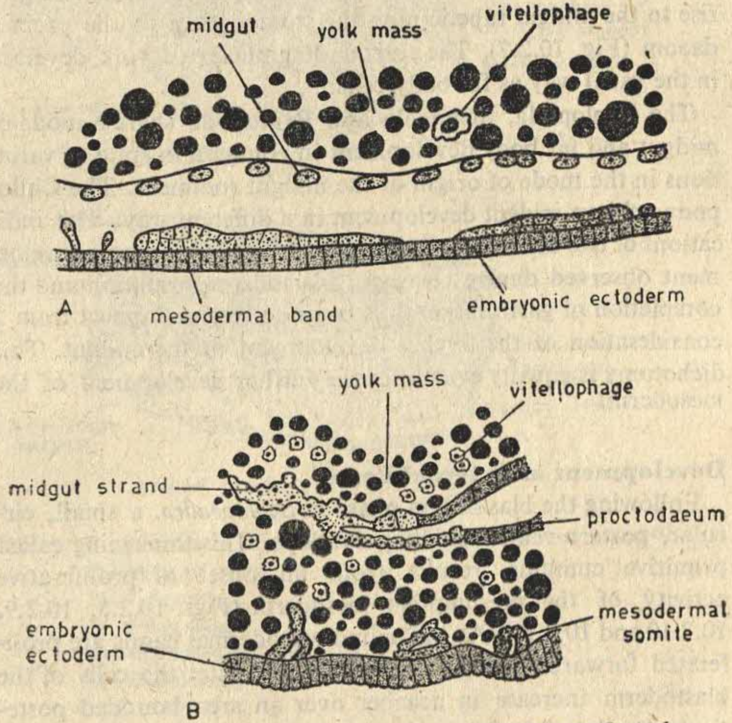


Fig. 10.2.6. A—T.S. through the early germ band of *Scolopendra*. B—Sagittal section showing early development of the midgut strand of *Glomeris*.

strand of cells which runs through the centre of the yolk mass from the stomodaeum to the proctodaeum. The strand hollows out and the cells increase in number and form an epithelium around the central lumen. The nucleated yolk mass lies entirely external to the developing midgut and transforms gradually into the cells of the fat body.

Midgut development in the Symphala and Pauropoda (Fig. 10.2.7) proceeds in an essentially similar way. The mesoderm of the sypmhalan, *Hansaniella*, develops so as to separate a central strand of nucleated yolk from paired lateral masses. The central strand differentiates into a midgut tube joining the stomodaeum to the proctodaeum. The lateral masses develop into fat-body cells. *Pauropus* has a distinct midgut rudiment in the form of two yolky cells in the centre of the embryo. These cells slowly divide thus increasing the number of cells, and give rise to the midgut tube joining the stomodaeum to the proctodaeum (Fig. 10.2.7). The surrounding nucleated yolk develops in the usual way as fat body cells.

The Diplopoda, Symphala and Pauropoda share a mode of midgut and fat body development in common in spite of variations in the mode of origin of the midgut rudiment. The Chilopoda achieve midgut development in a different way. The indications of two alternative modes of myriapod embryonic development observed during cleavage, blastoderm formation and the completion of gastrulation thus begin to receive support from a consideration of the further development of the midgut. This dichotomy is equally evident in the further development of the mesoderm.

Development of External Form

Following the blastoderm phase in *Scolopendra*, a small, circular, postero-ventral swelling develops. This thickening called primitive cumulus, results from an onset of proliferative activity of the presumptive mesoderm (Figs. 10.2.8, 10.2.9, 10.2.10 and 10.2.11). As the paired mesodermal bands are proliferated forwards ventro-lateral from this site, the cells of the blastoderm increase in number over an area bounded posteriorly by the site of mesoderm proliferation, laterally by the mesodermal bands and anteriorly by a transverse line anterior to the presumptive stomodaeum. As the cells become more

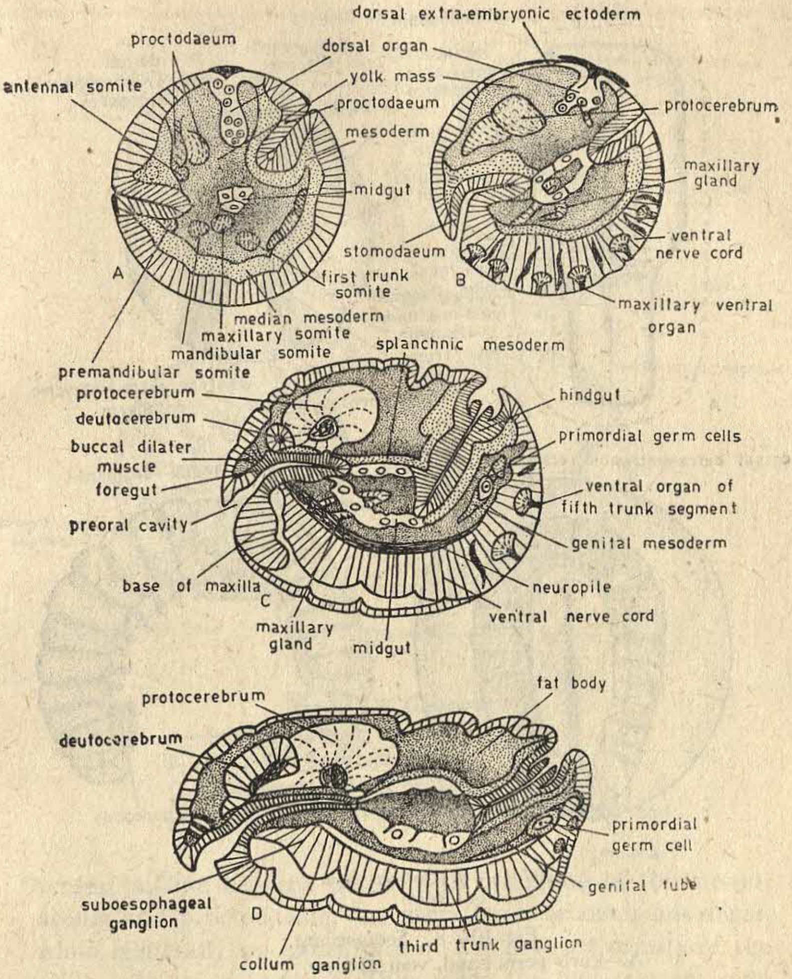


Fig. 10.2.7. Diagrammatic sagittal sections through the embryo of *Pauropus sylvaticus*.
A—7-day stage.
B—9-day stage.
C—Advanced embryo—11-day stage.
D—Pupoid stage.

numerous, the area can be distinguished externally as the germ band. The remainder of the blastoderm remains attenuated, forming the dorsal extra-embryonic ectoderm. The germ band

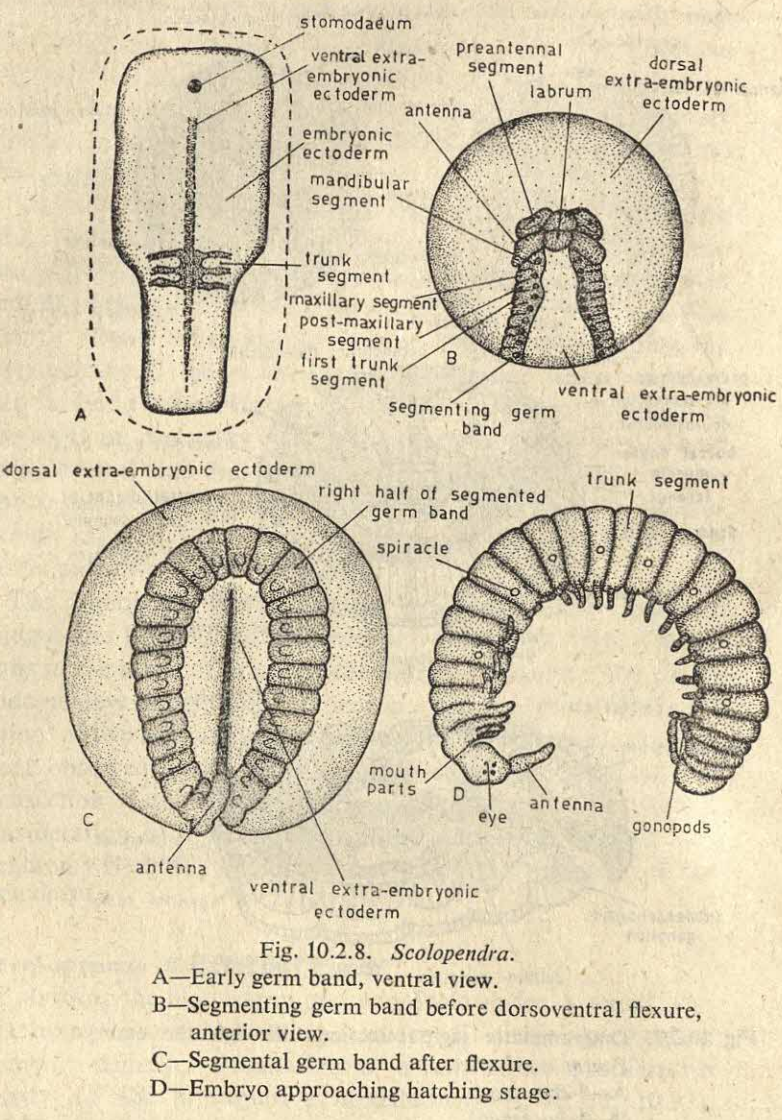


Fig. 10.2.8. *Scolopendra*.

A—Early germ band, ventral view.

B—Segmenting germ band before dorsoventral flexure, anterior view.

C—Segmental germ band after flexure.

D—Embryo approaching hatching stage.

is broad throughout its length, but is slightly broader anteriorly than posteriorly.

The onest of external delineation of segment begins before any further increase takes place in the length of the germ band and is accompanied by another significant change. Along the

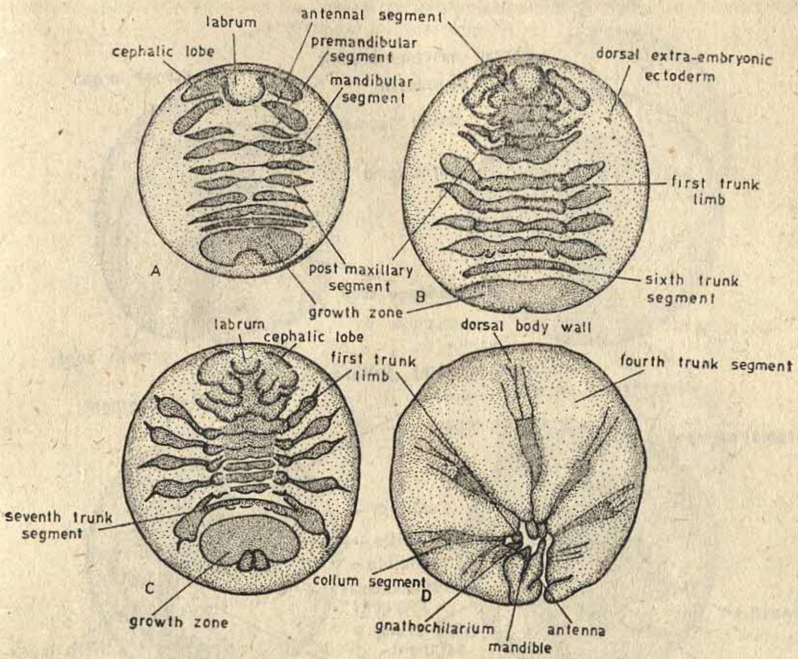


Fig. 10.2.9. *Glomeris*.
A—Segmenting germ band, ventral view.
B—Limb-bud formation.
C—Continued segment proliferation.
D—Embryo after dorso-ventral flexure, lateral view.

ventral midline between the sites of formation of the stomodaeum and proctodaeum, the germ band becomes less dense, while bilaterally on either side of this line, the density of the cells continues to increase. Thus when the first segment rudiments are delineated, they take the form of bilateral paired swellings on either side of a mid-ventral groove.

The first segments to become obvious lie in the middle region of the germ band on three pairs of short, wide swellings, the rudiments of trunk segments 11-13. Further segment delineation quickly follows. The cephalic and the first ten trunk segments are delineated simultaneously. A pair of small pre-antennal segment rudiments flanks the stomodaeal opening, leaving a short broad acron anteriorly. Paired, larger rudiments of the antennal

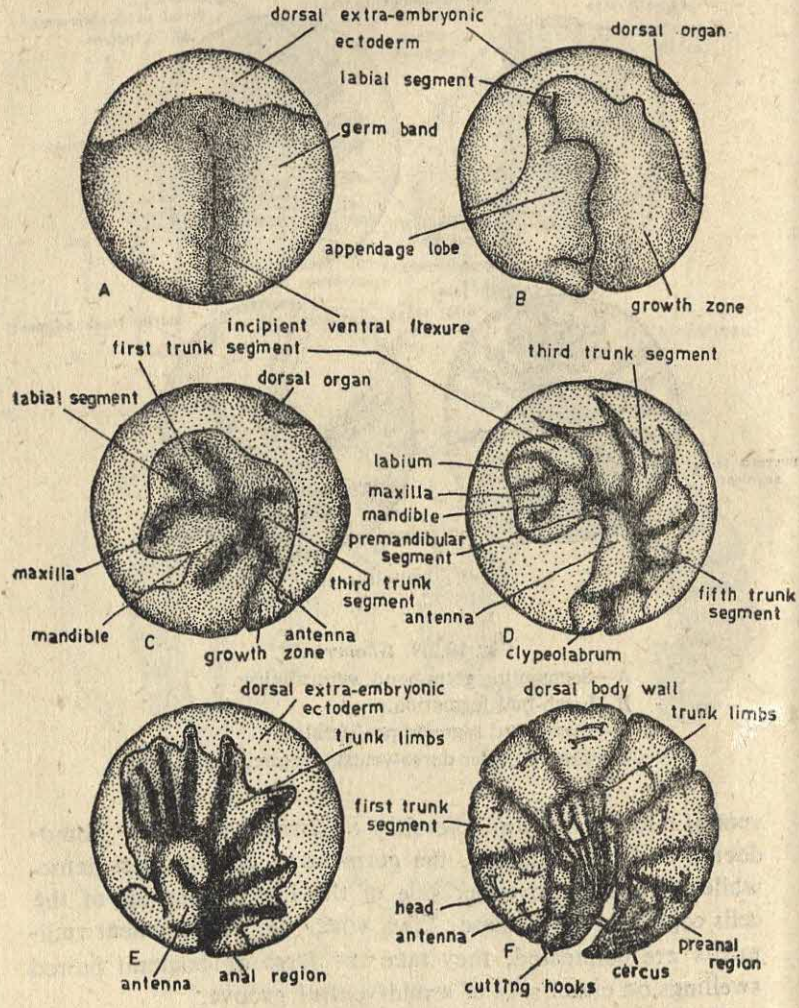


Fig. 10.2.10. *Hanseniella*.

A—Germ band showing formation of precocious ventral flexure.

B—5-day stage.

C—Early 6-day stage.

D—Late 6-day stage.

E—8-day stage.

F—Approaching hatching.

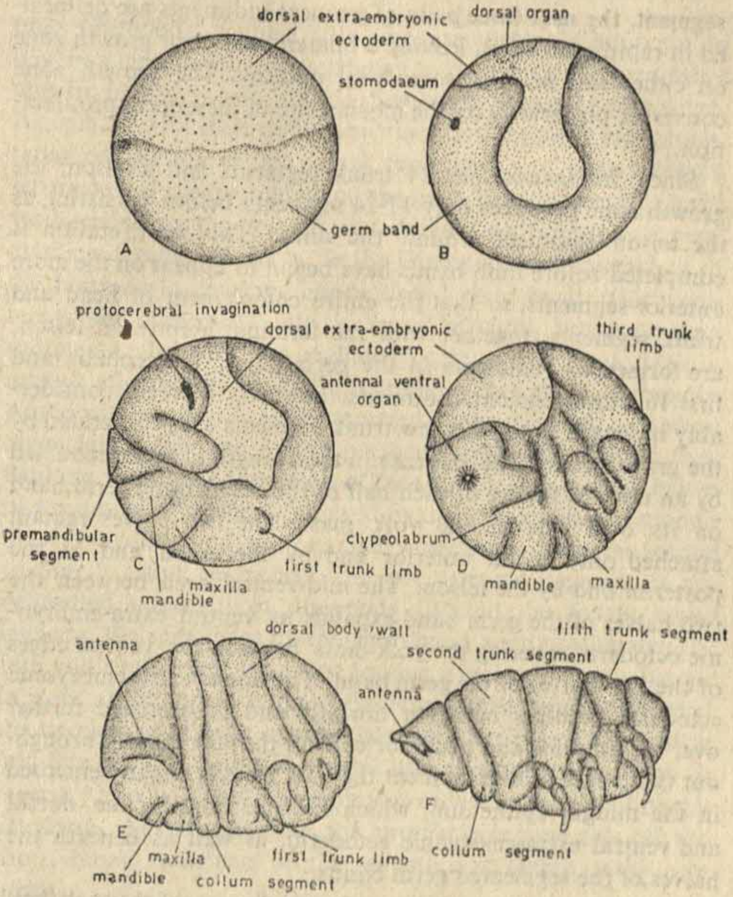


Fig. 10 2.11. *Pauropus.*

A—Differentiation of blastoderm

B—Germ band.

C—7-day stage.

D—9-day stage.

E—10-day stage.

F—Early pupoid stage with sheath removed.

segment lie behind the stomodaeal opening, followed by swollen mandibular, large maxillary and post-maxillary segments and a sequence of ten trunk segment rudiments. The pre-mandibular segment has not been identified externally, though it has the usual coelomic sacs and ganglia. Behind the thirteenth trunk segment, the next three pairs of segment rudiments are delineated in rapid succession, leaving a bilateral posterior growth zone on either side of the proctodaeal opening. The growth zone converges posteriorly on the median site of mesoderm proliferation.

Since *Scolopendra* has 24 trunk segments and a telson, the growth zone produces only 17-24 segments before persisting as the telson rudiment around the anus. This proliferation is completed before limb bands have begun to appear on the more anterior segments, so that the entire complement of head and trunk segments, together with the terminal acron and telson, are formed as rudiments in the germ band. The cephalic and first 16 trunk segment rudiments, however, increase considerably in length as the last few trunk segments are proliferated by the growth zone. The increase in total length is accommodated by an upward flexure of each half of the segmenting germ band on its own side of the yolk mass. The two halves remain attached only at the anterior end by the acron and at the posterior end by the telson. The mid-ventral area between the two halves of the germ band expands as ventral extra-embryonic ectoderm covering the yolk mass between the ventral edges of the two halves of the germ band. The dorsal extra-embryonic ectoderm becomes narrower dorsally and is stretched further over the anterior and posterior ends of the yolk mass. Throughout this phase of development the yolk mass is already enclosed in the midgut epithelium, which lies just beneath the dorsal and ventral extra-embryonic ectoderm, as well as beneath the halves of the segmented germ bands.

As the elongation and dorso-ventral flexure of the each half of the germ band is completed, the anterior and posterior ends, the acron and telson respectively move towards one another over the ventral surface of the yolk mass and come into close proximity in a mid-ventral position. This posture is retained during further development. The yolk mass shrinks, together with the extra-embryonic ectoderm, and the two halves of the body merge in the dorsal and ventral mid-line to complete the tubular body wall. Thus the long-bodied tubular embryo is accommodated within the ovoid egg space by being tightly dorso-ventrally flexed about its midpoint.

The Diplopoda, Symphala and Pauropoda and the anamorphic Chilopoda contrast with the epimorphic Chilopoda in having a smaller yolk mass relative to the size of the germ band and in hatching with only a few trunk segments completed. Associated with these differences they lack the early elongation, rapid completion of segmental formation and wide bilateral separation of the two halves of the germ band observed in the scolopendromorphs and do not develop an extensive, ventral extra-embryonic ectoderm. In the Diplopoda the ventral half of the blastoderm develops into a broad germ band which segments simultaneously into a series of short, broad segment rudiments. Initially the rudiments are paired and flank a narrow mid-ventral band of this tissue as they do in chilopods. Anteriorly an acron is demarcated, consisting of a median clypeolabral lobe and paired protocerebral lobes. Behind this, flanking the site of formation of the stomadaeum, are the halves of the antennal segment followed by the premandibular, mandibular, maxillary and post-maxillary segments, then the second to fourth trunk segments. The pre-antennal segment is not delineated externally in diplopods. Behind the fourth trunk segment, the remainder of the germ band soon proliferates the fifth and sixth trunk segments, leaving a posterior growth zone in front of the terminal anus. As development proceeds further the two halves of each segment concentrate towards the ventral mid-line and the thin mid-ventral area is eliminated. It can be regarded as a vestigeal representation of ventral extra-embryonic ectoderm. At the same time, the proliferative zone cuts off the short, broad rudiments of the seventh and eighth trunk segments.

Gross development of the Symphala (Fig. 10.2.10) shows further modification in relation to the reduction of the yolk mass. The embryonic primordium is at first a hemispherical cup of cells formed directly as blastoderm cells and covering slightly more than the ventral half of the yolk mass. Precociously, before segment delineation begins, the primordium undergoes a transverse dorso-ventral flexure which pulls the anterior and posterior ends downwards to meet each other in the mid-ventral position and flexes the entire ventral surface between them up into the yolk mass. At the same time, the dorsal extra-embryonic ectoderm is stretched over the anterior and posterior poles of the yolk. Segmentation of the germ band now takes place. The cephalic segments are delineated first. A median clypeolabral and paired protocerebral lobes lying in front of the stomodaeal opening are followed by postoral, antennal, premandibular, mandibular, maxillary and post-maxillary segments. The pre-antennal segment is not delineated externally but can be identified by other criteria. Behind the point of flexure, the first three trunk segments are delineated in rapid succession. They take up most of the posterior half of the germ band, leaving a segment-forming growth zone in front of the anus. The growth zone then gives rise to four further trunk segments and then divides into a large preanal segment, and a small anal segment on which the anus lies. The preanal 'segment' is more than a single segment, since it includes the growth zone from which all subsequent segments arise during anamorphic post-embryonic growth. The terminal unit, the anal-segment, presumably incorporates a vestigeal telson. As the fourth to the seventh trunk segments are formed the dorso-ventral flexure of the elongating embryo increases and the embryo becomes highly coiled within the spherical egg space. Lateral upgrowth of the body wall then completes dorsal closure, replacing the dorsal extra-embryonic ectoderm.

In the gross development of the Pauropoda, the embryonic primordium in the small eggs of *Pauropus* omits ventral flexure entirely (Fig. 10.2.11). The embryonic primordium formed at the blastoderm stage occupies all except the dorsal part of the spherical yolk surface. As it narrows and elongates into a germ band, the primordium remains curved around the yolk surface and the anterior and posterior ends approach one another in the mid-dorsal position. Associated with this, the small area of the dorsal extra-embryonic ectoderm is stretched down the sides of the yolk. The anterior end of the germ band, anterior to the stomodaeal opening develops as a median clypeolabral area and paired protocerebral lobes. There is no external expression of a pre-antennal segment, but a large antennal segment can now be recognized just behind the level of the stomodaeum. Behind this again is a small pre-mandibular segment followed in temporal succession by mandibular and maxillary segments, then the post-maxillary and second to fourth trunk segments. As in diplopods, the dorsal wall of the post-maxillary or first trunk segment forms the collum. The fourth trunk segment is delineated near the posterior end of the germ band leaving a terminal growth zone in front of and around the anus. While upgrowth of the lateral walls of the segments effects dorsal closure, the curved embryo straightens out. Short fifth and sixth trunk segment rudiments are then demarcated in front of the growth zone, but at hatching, only the first four trunk segments have attained the functional condition, as compared with the first seven in symphalans and eight in diplopods.

The very small egg of *Pauropus* thus develops with a greater degree of anamorphosis than the larger eggs of diplopods and Symphala. In the embryos of all the three classes the germ band includes the rudiments of the first nine serial segments and that all additional trunk segments stem from a posterior growth zone. Thus, the varying degrees of anamorphosis in these myriapods, attaining an extreme in the small embryos of *Pauropus*, are associated with different degrees of suppression of the activity of the growth zone in the embryo.

Development of Limbs

All myriapod embryos develop a pair of large, antennal limb buds, which are post oval in position when first formed. Behind them lie a pair of mandibular limb buds and a pair of maxillary limb buds only. *Scolopendra* has been found to have a pair of transitory preantennal limb vestiges in front of the antennae, on either side of the mouth. These limbs are not formed in other myriapods. Similarly only the symphalan, *Hanseniella* has been found to develop a pair of transient, pre-mandibular limb buds. Other myriapods lack these vestiges.

During the further development of the head (Fig. 10.2.12), the median clypeolabral lobe forms the antero-ventral surface of the head, including the labrum. The lateral, protocerebral lobes meet in the dorsal mid-line to form the dorsal wall of the head, and the pre-antennal, antennal and anterior margins of the pre-mandibular segment halves migrate forwards on either side of the mouth, to contribute to the lateral walls of the head and the undersurface of the labrum. As part of the forward migration, the antennae are carried into a preoral position and the mandibles come to flank the mouth with the maxillae just behind them ventrolaterally. The maxillae remain paired in

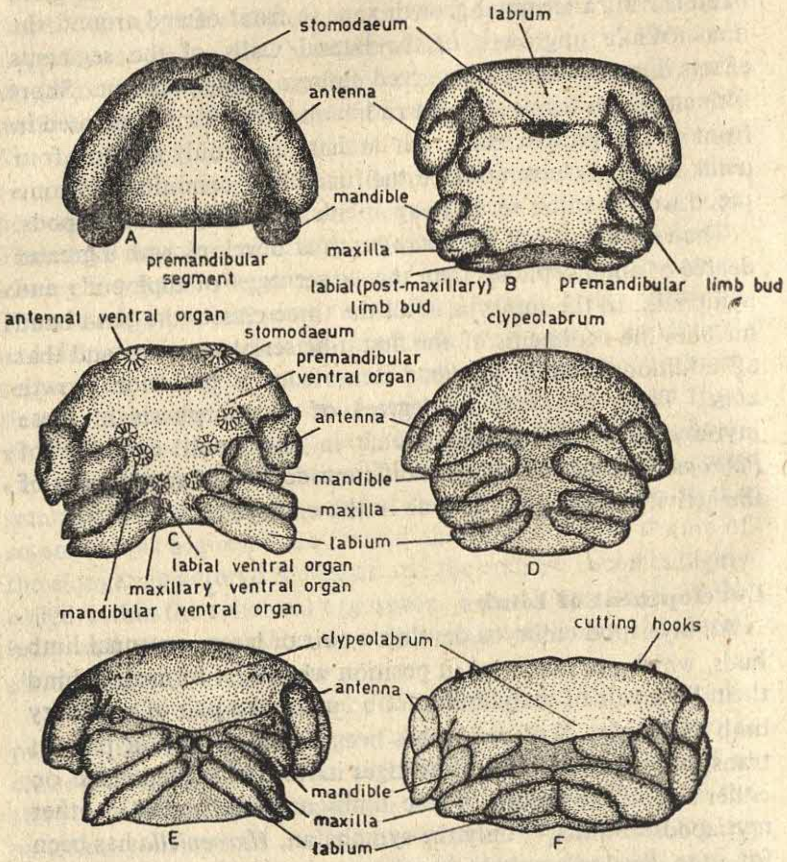


Fig. 10.2 12. External development of the head of *Hanseniella*, ventral view.

A—Early 7-day.
B—Late 7-day.
C—Early 8-day.
D—Late 8-day.
E—Early 9-day.
F—Late 9-day.

Chilopoda, Symphala and Pauropoda, but unite to form the gnathochilarium in the Diplopoda.

The developmental role of the post-maxillary segment differs in each group of myriapods. In chilopods this segment is incorporated into the head as the second maxillary segment.

but its limb buds develop in a generalized way and remain quite leg-like. The post-maxillary segment of the Symphala is also incorporated into the head, but shows a greater specialization of its limb buds, which unite and develop as the symphalan labium. The same segment in the Paurapoda and Diplopoda in contrast is the limbless first trunk segment, which gives rise to the collum. In those millipedes, in which the collum carries a pair of limbs, the limbs of the second to fifth trunk segments undergo a forward shift of one segment during later development.

Development of limb buds on the trunk segments of myriapod embryos varied with the degree of anamorphosis. *Scolopendra* develops a pair of limb buds on each trunk segment. The first pair on the seventh serial segment, transform into the specialized poison jaws, the next 21 develop as locomotory limbs and the last two pairs are rudimentary gonopods. In the diplopods, in contrast, only seven pairs of trunk limbs develop in the embryo, the last four pairs usually becoming grouped together as the pairs of the first two diplo-segments. The Symphala also develop seven pairs of functional trunk limbs in the embryo, but display no diplopody. Finally, in *Pauropus* only three pairs of trunk limbs are developed in the embryo, the remainder being added anamorphically after hatching.

Further Development of the Mesoderm

Mesodermal segmentation always precedes ectodermal segmentation. *Scolependra* shows the most generalized mode of mesoderm development among the myriapods (Fig. 10.2.13). In contrast to the pattern of external definition of the segments, the cells of the mesodermal bands group into paired somites in an anteroposterior sequence. The most anterior parts of the bands form a small pair of pre-antennal somites on either side of the site of presumptive stomodaeum, the acron being devoid of mesodermal components. The pre-antennal somites are followed by a large antennal pair, a small pre-mandibular pair and then a sequence of somites of more uniform intermediate size, those of the mandibular, first maxillary, second maxillary and trunk segments. The last few pairs of trunk somites are proliferated from the growth zone after the proctodaeum has been formed and move forwards on either side of the proctodaeum to com-

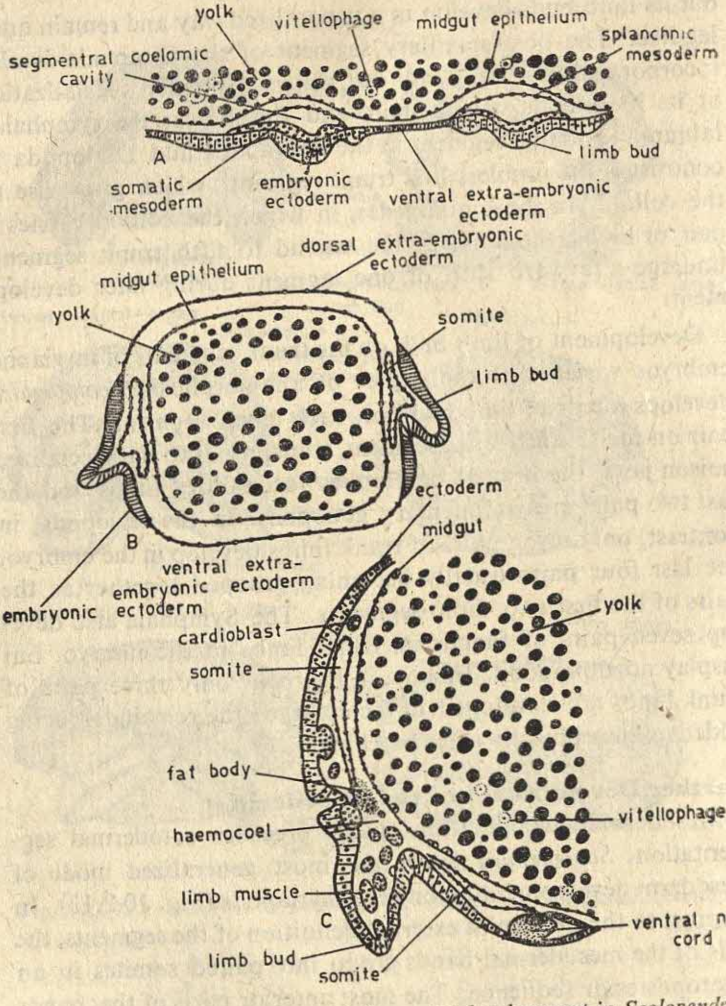


Fig. 10.2.13. T.S. through stages of segment development in *Scolopendra*.
A—Before dorsoventral flexure.
B—After flexure.
C—During dorsal closure.

plete the sequence of 22 pairs of trunk somites and two pairs of genital somites. The telson has no somites.

During the elongation and dorso-ventral flexure of each half of the germ band, the paired somites become widely separated from one another on either side of the yolk mass, especially in the middle region of the trunk. Each somite hollows out during this phase and develops a trilobed form. The somatic wall pouches out into the overlying limb bud as an appendicular lobe, while the remainder of the somite expands dorsally and ventrally to form dorsolateral and medioventral lobes beneath the embryonic ectoderm of the segment. The splanchnic walls of the dorsolateral and medioventral lobes are continuous and lie against the underlying midgut epithelium at the surface of the yolk mass.

During subsequent development, the pre-antennal and antennal somite pairs migrate forwards on either side of the stomodaeum as to attain a preoral position and the pre-mandibular somites come to lie on either side just behind the mouth. All pairs of cephalic and trunk somites, however, undergo the same major changes. As the yolk mass shrinks, the dorsolateral and medioventral lobes extend around the midgut towards the dorsal and ventral midlines. The appendicular lobes of the somites develop into limb musculature, except in the pre-antennal segment, whose limbs are transient, and the pre-mandibular segment, which lacks limb vestiges. The basal regions of the appendicular lobes release cells, which develop as the fat body. The dorsolateral lobe gives off cell masses from their somatic walls which develop as dorsolateral lobes then come together in the dorsal midline to form the heart and anterior aorta. All pairs of somites, including the pre-antennal and premandibular pairs contribute to the dorsal blood vessel. In the trunk, the somatic walls of the dorsolateral lobes also stretch out to form the pericardial septum, while beneath the septum, part of the coelomic cavity of each dorsolateral lobe is enclosed and becomes confluent with the others of its side forming a pair of genital tubes. The splanchic walls applied to the external surfaces of the midgut, develop as midgut musculature.

Development of the medioventral lobes begins in a similar way. The lobes extend towards the ventral midline, except for those anterior to the mandibular segment, and their edges come together to form the ventral blood vessel. The splanchnic walls develop as gut musculature and the somatic walls give off cells which develop as ventral longitudinal muscle. The remainder of the development of the medioventral lobes differ from that of the dorsolateral lobes, however, in that the coelomic cavities are lost and the somatic cells differentiate as transverse ventral muscles. The last two pairs of medioventral lobes, in the two genital segments, develop ventrolateral outgrowths in the form of coelomoducts, retaining coelomic internal cavities. The coelomoducts subsequently develop as genital ducts.

Certain peculiarities attend the development of the anterior cephalic somites. Obviously none of them contributes to the pericardial septum, gonads or ventral blood vessel. The somatic walls of the pre-antennal somites form, in addition to the anterior ends of the ventral longitudinal muscles, only clypeolabral musculature. The somatic walls of the antennal somites contribute, apart from antennal muscles and components of the anterior aorta, only ventral longitudinal muscle and somatic musculature of the head capsule. The somatic walls of the pre-mandibular somites are specialized in another way. Apart from their contribution to the anterior aorta and longitudinal muscles, the walls of these somites develop mainly as paired masses of lymphoid tissue in the head. The lymphoid masses are interpreted as the vestiges of a pair of pre-mandibular segmental organs.

The three pairs of postoral cephalic segments are of course, much more normal in development, especially the second maxillary pair. As compared with the trunk somites, they lack only the pericardial and genital components. None of these three somite pairs develops any trace of segmental organs.

When the mesodermal bands are first formed in early embryo, a scattering of mesoderm cells lies between them as median mesoderm. The median mesoderm is subsequently left in the ventral position while the somites form and separate. In the chilopods, the anterior mesodermal cells are carried inwards as a covering sheath on the ingrowing stomodaeum and proctodaeum and envelopes as stomodaeal and proctodael musculature respectively. The intervening median mesoderm gives rise to blood cells.

In the symphalan *Hansaniella* (Fig. 10.2.14), the early germ band develops a pair of lateral cords of mesoderm extending from the stomodaeal to the proctodaeal level, together with a sheet of median mesoderm between the lateral cords. Before external segmentation becomes apparent, the lateral cords pile

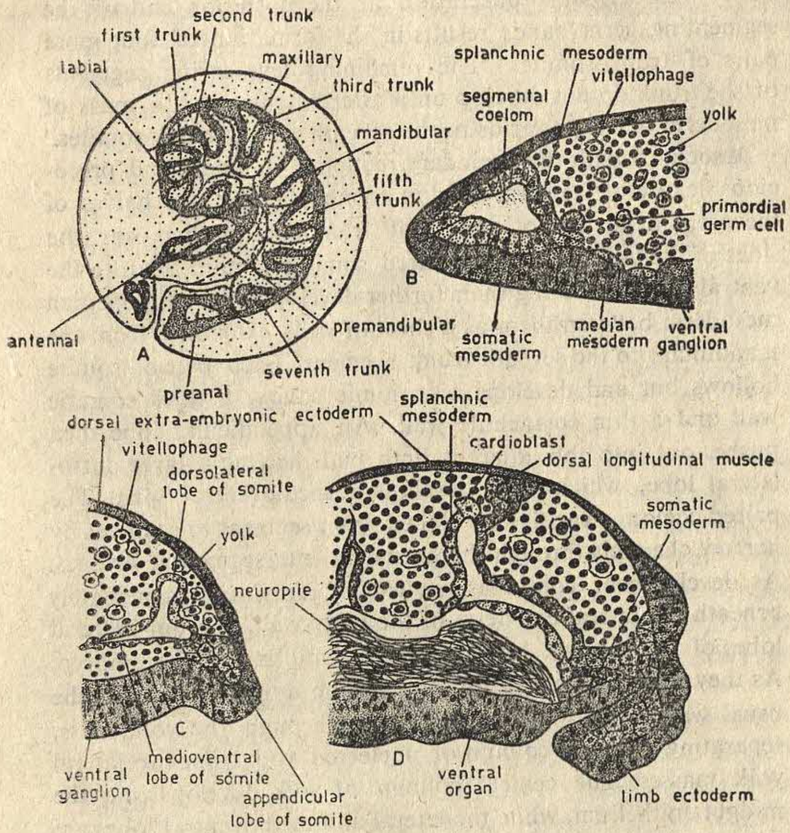


Fig. 10.2.14. *Hanseniella*.

A—Diagrammatic lateral view of 7-day embryo showing distribution of somites.

B—T.S. through 5th trunk segment of 7-day embryo.

C—T.S. through 5th trunk segment of early 8-day embryo.

D—T.S. through 6th trunk segment of late 8-day embryo.

up into paired somite masses, linked by narrow intersegmental bridges. Somite formation begins at the mandibular level, but spreads rapidly forwards and backwards. A pair of small pre-antennal somites is formed anterolateral to the stomodaeum, followed by a pair of large antennal somites behind the stomodaeum, then pre-mandibular, mandibular, maxillary and labial pairs and three pairs of trunk somites formed in rapid succession. Mesodermal proliferation at the posterior end of the segmenting germ bands results in the formation of four more pairs of trunk somites. The remaining leg-bearing segments of the trunk do not develop until after hatching, but a mass of mesoderm is left posterior to the seventh pair of trunk somites.

Associated with the secondary reduction in length and precocious flexure of the germ band in Symphala, the two halves of the germ band do not arch away from one another over the sides of the yolk mass. The paired somites remain near to the ventral mid-line during their further development, with median mesoderm between them as a sheet of cells extending from the mandibular to the seventh trunk segment. Each pair of somite hollows out and develops a coelomic cavity, a thick somatic wall and a thin splanchnic wall. An appendicular lobe then pushes out into the adjacent limb bud, leaving a large dorsolateral lobe, which soon develops a medioventral lobe. The paired coelomic cavities are linked from segment to segment by narrow channels through the persistent intersegmental bridges. As development continues, instead of remaining immediately beneath the lateral ectoderm of the body wall, the dorsolateral lobes of the somites shift towards the midline of the embryo. As they now grow upwards towards the dorsal surface in the usual way, these lobes cut vertically through the yolk mass, separating a central column of nucleated yolk from two lateral yolk masses. The central column of yolk develops into the midgut epithelium, while the lateral and medioventral lobes are adpressed to the central masses develop as fat body. The sphanchnic walls of the dorsolateral and medioventral lobes are adpressed to the central column of yolk as splanchnic mesoderm. In due course, the splanchnic mesoderm spreads ventrally beneath the developing midgut epithelium and develops as splanchnic muscle in the usual way.

Even though they shift inwards at an early stage towards the midline, the somatic walls of the dorsolateral lobes of the trunk somites of *Hanseniella* complete their development in essentially the same way as in chilopods. At their dorsal edges, these walls develop cardioblasts which come together from either side above the midgut and give rise to the heart. Dorsolaterally, cell masses are given off which differentiate as dorsal longitudinal muscles and tergosternal muscles. The remainder of the wall in each segment thins out, with obliteration of the coelomic cavity, and forms the pericardial septum. The genital tubes, however, are not formed from the dorsolateral lobes.

The appendicular lobes of the trunk somites of *Hanseniella* also develop in the same manner as in chilopods, giving rise to the musculature of the limb and limb base. A major difference is observed in the origin of fat body not from these cells, but from the lateral yolk masses above the pericardial septum.

Finally, the medioventral lobes show some similarities and some differences as compared with those of chilopods. The lateral parts of the medioventral lobes retain their coelomic cavities and give rise to the paired ventrolateral gonads. Germ cells differentiate in those parts of the genital tubes derived from the fifth to seventh pairs of trunk somites. Adjacent to their gonad-forming regions, the somatic walls of the medioventral lobes of the third to seventh pairs of trunk somites grow out towards the limb bases as ventrolateral coelomoducts, but these are only transient vestiges. Mesodermal genital ducts opening to a posterior, opisthogoneate gonopore are not developed in the Symphala. Instead, secondary ectodermal gonoducts are developed in an anterior, progoneate position during postembryonic development.

The pre-antennal and antennal pairs migrate forwards in front of the mouth, the premandibular pair comes to lie on either side of the mouth, and all pairs retain much of the generalized pattern of development observed in the trunk segments. Each pair of cephalic somites also has its own specializations. The labial somites are the least modified. They undergo the typical trilobed outpouching and develop the typical splanchnic mesoderm, cardioblasts, ventral longitudinal muscle and ventral sternal muscles, and extrinsic and intrinsic limb muscles previously seen in the trunk. Vestigial coelomoducts are absent, as are dorsal longitudinal muscle, pericardial septum and genital tube components. None of the last three structures extends into the head.

The maxillary somites are more specialized. The splanchnic wall separates off and grows up through the yolk in the usual way with cardioblasts at its dorsal edge, to contribute to the splanchnic musculature and heart. The somatic wall mainly breaks up directly into maxillary muscles and ventral longitudinal muscles; but, in addition, a small portion of the coelom median to the base of the limb remains enclosed in an epithelial sac. From these rudiments, a pair of coiled, maxillary salivary glands with small coelomic end sacs is developed. They acquire openings directly into the pre-oral cavity, with no ectodermal exit ducts, and are interpreted as coelomoduct segmental organs of the maxillary segment.

The mandibular somites, although large, with a spacious coelom, display a greater specialization than the maxillary somites. The splanchnic wall separates off in the usual way and contributes to the splanchnic musculature and heart, but the remainder of the somite becomes mesenchymatous and develops almost entirely as a mandibular and ventral longitudinal muscle. The only other product is a few cells which separate from the main mass as a pair of paracardial glands of uncertain function and homologies.

The pre-mandibular somites are the most specialized of all. The segment is mainly vestigial and has only transient limb buds. The somites are small and develop almost exclusively into a pair of coiled, pre-mandibular segmental organs with small end sacs. These organs, unlike those of the maxillary segment, acquire short, ectodermal exit ducts just lateral to the pre-ectodermal exit ducts just lateral to the pre-mandibular ganglia. The pre-mandibular segmental organs are large and conspicuous in the embryo, but degenerate and disappear shortly after hatching has taken place. Some of the cells of the pre-mandibular somites spread back into the first three trunk segments and differentiate as glandular nephrocytic organs. Unlike the pre-mandibular segmental orders, the nephrocytic organs persist into the adult.

The antennal somites, in contrast to the premandibular pair, are remarkably unspecialized, being typically large and trilobed, but their later products are somewhat aberrant. The appendicular lobe develops, of course as antennal muscle, but the medioventral lobe spreads over the dorsal wall of the stomodaeum and develops as the major part of the anterior aorta and the musculature of the stomodaeum. The dorsolateral lobes are more specialized, since they spread thinly beneath the large protocerebral gangila above them and develop as the neurilemma of the brain.

Finally, the pre-antennal somites lying in front of the stomodaeum in the developing clypeolabrum, are small and vestigial. Although transiently hollow, they soon break up into small clumps of mesenchyme, from which arise the clypeolabral muscles above the anterior end of the stomodaeum and also the anterior end of the anterior aorta.

In the Diplopoda, the ventrolateral mesodermal bands segregate into paired hollow somites joined by median mesoderm in the usual way, but the somites are small, and have rather thin walls. Eight pairs of somites are formed in the trunk, the first three being formed simultaneously with the cephalic somites, the remainder being segregated in succession from the proliferating growth zone mesoderm. In the head, paired antennal, small pre-mandibular, mandibular and maxillary somites remains continuous across the head lobes and intervening clypeolabral rudiment. There is no external indication of pre-antennal segment in diplopods.

The first pair of trunk somites, belong to the apodous collum segment, is vestigial and contributes only to the musculature of the gnathochilarium. The second and succeeding trunk somites develop typical appendicular lobes, together with medioventral lobes, and it is generally reported that dorsolateral lobes do not form. The mode of development of splanchnic mesoderm, heart, pericardial septum and dorsal longitudinal muscles is still not clear. Presumably these structures arise from the dorsal walls of the so-called medioventral lobes. The gonads definitely develop from the medioventral lobes, with germ cells first becoming apparent in the eighth and ninth pair of somites but no coelomoduct gonoducts are formed. The genital ducts arise as in Symphala, as secondary ectodermal ingrowths in the progoneate position. The development of the ventral longitudinal muscles, ventral blood vessel, blood cells, stomodaeal mesoderm and proctodaeal mesoderm is still not clear.

The maxillary pair of somites, in addition to playing a major part in the formation of gnathochilarial muscles, give rise to a pair of large segmental organs which differentiate as salivary glands. The mandibular pair develops as mandibular musculature and the antennal pair as antennal musculature. Pre-antennal somites are not formed and the labral musculature develops from the generalized mesoderm in front of the antennal somites.

In the small embryos of *Pauropus* (Fig. 10.2.15) although the somites are relatively vestigial, a distinct pair is formed in each of the six trunk segments developed in the embryo and also in the pre-antennal, pre-mandibular, mandibular and maxillary segments of the head. The somites arise in the usual way, by the break up of the paired ventrolateral bands of mesoderm formed in the germ band. The cephalic and first four trunk somites are formed directly. The fifth and sixth trunk somites are proliferated from posterior growth zone mesoderm. The somites remain near the ventral midline during their subsequent development and do not become separated from one another bilaterally.

The further development of mesoderm in *Pauropus* is specialized in various ways and much of the mesoderm has escaped from the somite route of development. In addition to the median mesoderm lying between the somites, continuous cords of mesoderm are left dorsolateral to the somites. From these cords, the dorsal longitudinal muscle is formed by direct development. The median mesoderm gives rise not only to the neurilemma of the nerve cord, but also to the stomodaeal muscle layer, the splanchnic mesoderm of the midgut and the genital tube. The only mesodermal development retaining the somite route in the trunk is that of the limb musculature and ventral longitudinal muscles. In the limbless collum segment even this contribution is severally reduced and the somites are very small and lacking in coelomic cavities. None of the trunk segments develop coelomoducts, the gonaducts being formed as ectodermal ingrowths in the progoneate position.

Similar reductions are obtained in the development of the cephalic somites of *Pauropus*. These somites cannot contribute to dorsal blood vessel, splanchnic mesoderm or dorsal longitudinal muscles. However, the maxillary somites release cells which develop as limb muscle and ventral longitudinal muscles at the maxillary level as in the trunk and then elongate and differentiate as a pair of maxillary segmental organs which become maxillary salivary glands. The mandibular somites break up and develop as mandibular and ventral longitudinal muscles. The pre-mandibular somites elongate and differentiate into a pair of pre-mandibular segmental organs with short ectodermal exit ducts. The pre-mandibular segmental organs of *Pauropus*

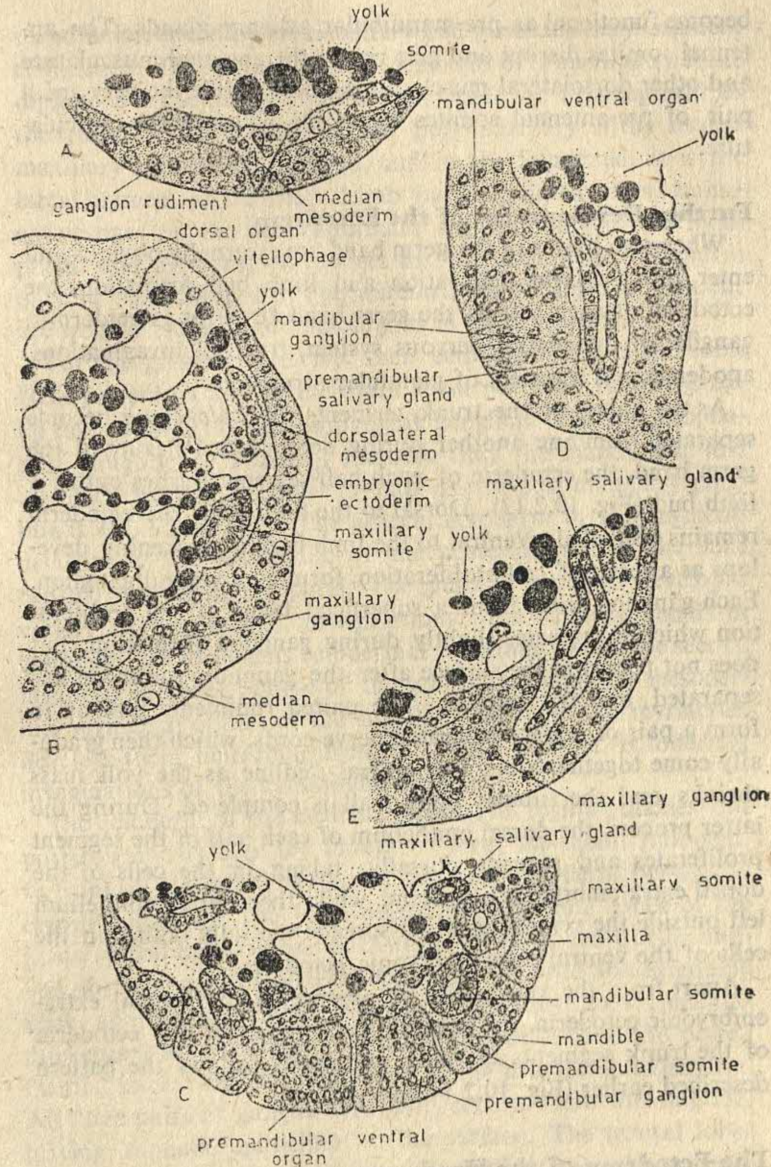


Fig. 10.2.15. T.S. showing segment development of *Pauropus*.
A—Maxillary segment of early 7-day embryo.
B—Maxillary segment of late 7-day embryo.
C—Mandibular segment of early 8-day embryo,
D—Mandibular segment of late 8-day embryo.
E—Maxillary segment of late 8-day embryo.

become functional as pre-mandibular salivary glands. The antennal somites disrupt and give rise to the antennal musculature and other dorsolateral muscles in the head, while the very small pair of pre-antennal somites forms the clypeolabral musculature.

Further Development of the Ectoderm

When two halves of the germ band are externally obvious and enter into segment delineation and limb bud formation, the ectoderm of each half of the germ band develops as epidermis, gangila of the central nervous system, tracheal invaginations, apodemes and a variety of glandular structures.

As the halves of the trunk segments of *Scolopendra* become separated from one another during dorsoventral flexure of the germ band, the ectoderm of each half-segment pushes out as a limb bud (Fig. 10.2.13). Dorsal to the limb bud, the ectoderm remains epithelial. Ventral to the limb bud, a thickening develops as a result of cell proliferation, forming a ventral ganglion. Each ganglion arises from a superficial focus of cell proliferation which invaginates slightly during ganglion formation, but does not persist at the surface after the ganglion rudiment has separated into the interior. The paired rudiments link up to form a pair of widely separated nerve cords, which then gradually come together along the ventral midline as the yolk mass shrinks and the tubular body wall is completed. During the latter process, the dorsal epithelium of each half of the segment proliferates and spreads dorsally, taking in the cells of the dorsal extra-embryonic ectoderm, while the ventral epithelium left outside the ventral ganglion spreads ventrally taking in the cells of the ventral extra embryonic ectoderm.

Apart from the vestigiality or absence of the ventral extra-embryonic ectoderm, the general development of the ectoderm of the trunk segments of other myriapods follows the pattern described earlier (Fig. 10.2.14 and 10.2.15).

The Ectoderm of the Head

As already seen, the embryonic head in myriapods comprise a median clypeolabral lobe flanked by broad protocerebral lobes in front of the mouth, a pair of pre-antennal segment rudiments just behind the mouth and a sequence of paired antennal, pre-mandibular, mandibular, maxillary and post-maxillary segment rudiments. The post-maxillary segment of *Pauropus* and the diplopods is the limbless collum segment, the first segment of the trunk. In the Chilopoda, the post-maxillary is the second maxillary segment of the head, and in the Symphala, it is the labial segment of the head. Limb buds are developed as transient vestiges on the pre-mandibular segment only in the Symphala, and as transient vestiges on the pre-antennal segment only in the Chilopoda. The antenna, mandibles and maxillae are well developed and homologous in all myriapods.

For the gnathal segments, their ectoderm follows the same basic pattern as that of the trunk segments. The ventral ganglia fuse to form the suboesophageal ganglion. The suboesophageal ganglion of *Pauropus* includes only the mandibular and maxillary ganglia. In other myriapods, the post-maxillary ganglion is also added to it. The general ectoderm of the segments develops as the epithelium of the posterior part of the head capsule and the mouth parts. The gnathal segments develop a variety of ectodermal invaginations. The salivary glands of chilopods are formed as ectodermal invaginations on the second maxillary segment. Those of the other myriapods are mesodermal segmental organs of the maxillary segment. All myriapods develop their tentorium from a single pair of ectodermal invaginations of the mandibular segment.

The ectoderm of the median clypeolabial region pouches out posteroventrally over the mouth to form the labral rudiment. In front of the labral rudiment, the same ectoderm persists as clypeal ectoderm. The large paired protocerebral lobes of the acron spread upwards and backwards towards the dorsal midline, forming the dorsolateral regions of the head and extending back above the gnathal segments. As they do so, cellular thickenings are proliferated into the interior to form the paired frontal, lateral and posterior lobes of the protocerebral ganglia. All three pairs of ganglia eventually separate into the interior, leaving cephalic epithelium at the surface. The frontal lobes become united in the anterodorsal midline, above the stomodaeum.

As the protocerebral lobes extend upwards and backwards, the pre-antennal, antennal and pre-mandibular segments move forwards on either side of the mouth to occupy the sites of the pre-oral region. The small preantennal segment develops a pair of distinct pre-antennal ganglia with associated central organs in Pauropoda. As the pre-antennal ganglia becomes internal, they merge with the frontal lobes of the protocerebral ganglia, but in *Pauropus*, the pre-antennal components can still be distinguished in the fully developed brain. The large antennal segment which makes up most of the lateral walls of the anterior part of the head, buds out typical limb buds before forward migration. Ventral to the limb buds, a pair of antennal ganglia is proliferated in the usual way, with associated ventral organs in the Pauropoda and Symphala. The ganglia become internal after they have moved forwards on either side of the mouth and lie on either side beneath the lateral lobes of the protocerebral ganglia. They become connected with the ipsilateral frontal lobes of the protocerebrum and are also joined transversely with one another by a preoral commissure. Once internal, the antennal ganglia become the deutocerebral ganglia.

The pre-mandibular areas of segmental ectoderm can be identified in the embryo mainly as the site of origin of the pre-mandibular ganglia. Ventral organs are again associated with these ganglia and provide important evidence of the distribution of pre-mandibular ectoderm after the ganglia of the segment have become internal. The pre-mandibular ganglia are joined anteriorly to the antennal ganglia and come to lie ventrolaterally, first anterior to the mouth, as the tritocerebral ganglia of the brain. They retain a post-oral transverse connection, as a tritocerebral commissure as well as being connected longitudinally with the suboesophageal ganglion. The forward migration of the superficial pre-mandibular ectoderm continues as the pre-mandibular ganglia become internal. Much of the superficial tissue spreads medially in front of the mouth and contributes to the floor of the preoral cavity.

Unlike the ventral organs of the gnathal segments, those of the pre-antennal, antennal and pre-mandibular segments merge without trace into the surface epithelium of the head. None of these segments produces any major ectodermal invaginations, though the protocerebral ectoderm develops a pair of organs of *Tomosvary* adjacent to the lateral lobes of the protocerebrum, just behind the bases of the antennae. A similar formation of these characteristic myriapod sense organs have been observed in the Chilopoda, Diplopoda, Symphala and Pauropoda.

The Dorsal Organ

The broadly alterated dorsal extra-embryonic ectoderm of the chilopod embryo has a uniform structure and fate and is gradually incorporated into the definitive dorsal epithelium of the body wall. In the other myriapods, however, it includes a localized mid-dorsal region, which has a different form and fate. This is the dorsal organ and is first developed when the dorsal extra-embryonic ectoderm becomes distinct from the embryonic primordium.

Hanseniella develops an elaborate dorsal organ. A circular patch of 20-30 dorsal blastoderm cells enters into cell elongation. The cells penetrate deeply into the underlying yolk mass and the external ends of the cells become narrow, so that the cells take on a flask-shaped form. External to the narrow ends of the cells, an aperture is left in the newly secreted blastodermal cuticle. The necks of the dorsal organ cells now become fibrillated. As the segmentation of the germ band takes place, thin filaments begin to grow out through the narrow necks of the cells and radiate into the space between the blastodermal cuticle and the chorion. In two days, the filaments have reached the ventral pole of the egg. The cells of the dorsal organ shrink and flatten as the threads are produced. Subsequently the cells gradually degenerate, but the threads remain in position until the embryo hatches. In *Pauropus* also a dorsal organ develops in a similar fashion but it sinks into the underlying yolk mass and gradually disintegrates.

Hatching

The symphalan embryos hatch directly, through rupture of the enclosing chorion and blastodermal cuticle, and become immediately active, but in other myriapods, the embryos hatch in two stages. As the embryo develops, an embryonic cuticle is secreted. In centipedes, the chorion ruptures before development is complete and the late embryo is released as a motionless 'pupoid' phase, enclosed in the embryonic cuticle. The active first larva escapes from this after a further period of development. Similar preliminary hatching as a 'pupoid' phase is well known in diplopods and pauropods.

The Basic Pattern of Development in Myriapoda

As was already seen in the preceding pages, the embryology of modern Myriapoda can be classified into two discrete groups; the Chilopoda on the one hand and the Symphala, Pauropoda and Diplopoda on the other. Furthermore, there are also indications that the Pauropoda and Diplopoda together differ in certain ways from the Symphala. In order to test the possibility of a basic theme in myriapod development, it is necessary to summarize these resemblances and differences, in terms of the formation and fates of presumptive areas of the blastoderm.

1) The eggs of Chilopoda are large, yolky, up to 3 mm in diameter, laid by oviparous female. Cleavage is intralecithal and results in the formation of uniform blastoderm. Some of the cleavage energids remain within the yolk mass as vitellophages which are subsequently resorbed with the yolk.

The Symphala, Pauropoda and Diplopoda are also oviparous, but lay eggs of smaller dimensions, cleavage is total at first proceeding in a specialized manner through the formation of yolk pyramids. Radial mitotic divisions separate an inner mass of yolky cells from a superficial layer which forms blastoderm.

2) In Symphala and Pauropoda, the blastoderm exhibits precocious differentiation of an embryonic primordium. The inner mass of cells fuses to form a yolk mass containing vitellophages. The fat body develops from vitellophages during later development. In the Chilopoda on the other hand, the fat body originates from the mesoderm somites.

3) The chilopod presumptive midgut is broadly spread throughout the ventral blastoderm. Numerous cells immigrate beneath the blastoderm, spread around the yolk mass and develop as the midgut epithelium. Additional vitellophages invade the yolk mass from a small posteroventral area behind the presumptive proctodaeum. This area subsequently becomes the site of proliferation of the mesodermal bands. The diplopod presumptive midgut is localized as a small blastodermal area in front of the presumptive proctodaeum. From this site, cells are proliferated as a strand through the centre of the yolk mass and

develop directly into the midgut epithelium. The Pauropoda exhibits a precococious segregation of the same cells in the centre of the embryo during cleavage and has no blastodermal representation of presumptive midgut. The Symphala also develop their central midgut strands from cells which are cut off into the interior during cleavage, but in a different way. Initially, all of the internal cells cut off during cleavage become vitellophages within the yolk mass. A subsequent separation of the central vitellophages as midgut and lateral vitellophages as fat body is then brought about by the mesoderm.

4) The stomodaeum develops in a constant manner in all myriapods, originating from a small presumptive area situated in the ventral midline of the blastoderm, between the presumptive protocerebral ectoderm and pre-antennal ectoderm. The stomodaeal rudiment invaginates independently and develops as foregut. The proctodaeum, originating from a small presumptive area situated immediately in front of the presumptive mesoderm in chilopods and in an equivalent position in other myriapods, also invaginates independently and develops into the hindgut and Malpighian tubules.

5) The mesodermal bands of chilopods for most part are proliferated forwards from a small, posteroventral area of presumptive mesoderm behind the presumptive proctodaeum. Additional cells migrate into the mesodermal bands from overlying blastoderm. Ventrally between the mesodermal bands, similar immigrating cells form scattered median mesoderm. The mesodermal bands extend anteriorly as far as the presumptive stomodaeum and include the mesoderm of twenty-two serial segments and a posterior growth zone. The symphalan, pauropod and diplopod mesoderm arises entirely through diffuse immigration of ventral blastoderm cells into the interior, mainly ventrolaterally as paired bands extending from the stomodaeal to the proctodaeal level, but also as median mesoderm between them. The mesodermal bands include the mesoderm of nine serial segments and a posterior growth zone.

In the further development of the mesoderm of all myriapods, the pre-antennal and antennal somites migrate to a pre-oral position and the pre-mandibular somites come to lie on either side of the mouth. The mandibular and maxillary somites are always cephalized post-orally, but the post-maxillary somites vary. In chilopods and symphalans they become part of the head but retain a generalized form and development. In pauropods and diplopods, the post-maxillary somites are reduced in association with specialization of the post-maxillary segment as the limbless collum at the anterior end of the trunk.

6) The chilopod trunk somites become partially subdivided into three compartments, dorsolateral, medioventral and appendicular. The appendicular compartment pushes out as a lobe which gives rise to limb musculature and basally to fat body cells. The dorsolateral compartment extends towards the dorsal midline. Its somatic wall gives rise to dorsal longitudinal muscle, pericardial floor, gonad and heart components, while its splanchnic wall forms midgut musculature. The medioventral compartment extends towards the ventral midline. Its somatic wall gives rise to ventral longitudinal and transverse muscle and marginally to ventral blood vessel, while its splanchnic wall again forms midgut musculature. The gonoducts arises as coelomoduct outgrowths of the medioventral compartments of the last two trunk segments. Coelomoducts are absent from the other trunk somites. The chilopod median mesoderm gives rise to blood cells and, anteriorly and posteriorly to the stomodaeal and proctodaeal mesoderm.

The development of the somites of symphalan embryos concurs in general with that of the chilopods, but there are certain fundamental differences. The dorsolateral lobes shift towards the midline and cut upwards through the yolk mass, separating central midgut from lateral fat body. The appendicular lobes of the symphalan somites have no fat-body component. The gonads develop from the medioventral lobes by the trunk somites and these lobes, in several somites also develop transient coelomoducts. The definitive gonoducts are secondary ectodermal ingrowths. The ventral blood vessel develops from median mesoderm, while the blood cells are given off by the dorsolateral lobes of the somites. The stomodaeal and proctodaeal mesoderm also have a somatic origin, antennal anteriorly, preanal and anal posteriorly. The symphalan median mesoderm, in addition to forming the ventral blood vessel, also forms the neurilemma of the ventral nerve cord, which in chilopods develops from ectoderm.

The further development of the diplopod mesoderm is not well understood and that of the pauropod mesoderm is specialized in many ways, but several significant points can be made. Diplopods are said to lack the dorsolateral lobes of the somites. The mesoderm grows dorsally from the trunk somites, between the central midgut strand and lateral masses of fat body cells, to form the splanchnic mesoderm of the midgut, the heart and the dorsal longitudinal muscles. The gonads develop from the medioventral lobes of the trunk somites, although no coelomoducts have been reported even as vestiges, in the trunk. The gonoducts arise as secondary ectodermal ingrowth. Development of the ventral blood vessel and formation of the blood cells have not yet been described, but the stomodaeal and proctodaeal mesoderm arise from median mesoderm. The origin of the neurilemma in diplopods is not yet known. In the head, the fate of the pre-mandibular somites is still unknown but it is clear that they do not form segmental organs. The maxillary somites, however give rise to a pair of segmental organs which develop as salivary glands.

Pauropod trunk somites lack dorsolateral and medioventral lobes and give rise only to muscles. The dorsal longitudinal muscles have a specialized, extra-somitic origin. The heart and pericardial floor are absent and the splanchnic mesoderm of the midgut is proliferated from the terminal splanchnic mesoderm of the stomodaeum, clearly a specialization. The single medioventral gonad develops from median mesoderm, a second specialization. Coelomoducts are absent and the gonoducts arise as secondary ectodermal ingrowths. There is no ventral blood vessel and the origin of the few blood cells is unknown. The stomodaeal and proctodaeal mesoderm and the neurilemma of the ventral nerve cord arise from median mesoderm. In the head, both the pre-mandibular and maxillary somites give rise to segmental organs, and both pairs develop as salivary glands. The somites of the chilopod head are variously modified. The three post oral pairs lack only the gonad and pericardial septum components, but the pre-oral pairs are further reduced. They make no contribution to the splanchnic musculature, ventral blood vessel or fat body. The antennal somite retain appendicular lobes but the small pre-antennal and pre-mandibular somites lack these components. Like the trunk somites, the cephalic somites of chilopods develop no coelomoducts, but the pre-mandibular somites develop in part as lymphoid tissue.

On present evidence, the further development of the mesoderm of the Symphala, Diplopoda and Pauropoda can be seen to have a common ground retained in its most generalized form in the Symphala. A minor difference between the Symphala on the one hand and the Diplopoda and Pauropoda on the other is seen in the mode of formation of the stomodaeal and proctodaeal mesoderm. The further development of the mesoderm of the Chilopoda, on the other hand, proceeds in a manner fundamentally different in many ways from that of other myriapods.

In all classes of Myriapoda, except Chilopoda, the presumptive ectoderm comprises a broad band of ventral blastoderm cells, left at the surface after the immigration of mesoderm into the interior. The remainder of the blastoderm, dorsally to laterally, makes up to the dorsal extra-embryonic ectoderm. A midventral band of ventral extra-embryonic ectoderm develops between the presumptive stomodaeum and proctodaeum and become extensive during the later elongation and separation of the two halves of the germ band.

The presumptive ectoderm of the epimorphic chilopods includes the rudiments of the cephalic and first sixteen trunk segments as well as a posterior growth zone. The latter proliferates the ectoderm of the remaining trunk segments in the embryo and then gives rise to the telson. Following segment delineation, the segmental ectoderm pouches out ventrolaterally as limb buds and thickens ventrally as segmental ganglia. The chilopod ventral ganglia develop by proliferation from invaginated pits of ectoderm but lack associated ventral organs. A median strand of ectoderm developed between the ganglia contributes the neurilemma of the ventral nerve cord. A short, midventral invagination of ectoderm in front of the anus gives rise to the opisthogoneate gonopore. The salivary glands are formed as ectodermal invaginations on the second maxillary segment, the tentorial arms as ectodermal invaginations on the mandibular segment. Apodemes and tracheae are also ectodermal invaginations. The dorsal extra-embryonic ectoderm of chilopods does not develop a dorsal organ.

The presumptive ectoderm of the Symphyla, Pauropoda and

Diplopoda includes the rudiments of the cephalic and first four trunk segments, together with a posterior growth zone. The symphalan growth zone adds the rudiments of the next four trunk segments, the preanal segment and the anal segment in the embryo. The diplopod growth zone adds the ectoderm of at least four further segments in the embryo and persists behind the last of these, in front of the anus. The pauropod growth zone acts in a similar way to that of the diplopods, but adds the rudiments of only two further segments in the embryo.

The further development of the segmental ectoderm, the ventral ganglia of the Symphala and Pauropoda develop in association with ventral organs and there are hints of a similar mode in the Diplopoda. The ventral organs of the trunk segments in the Symphala and Diplopoda subsequently transform into exsertile vesicles on the trunk segments. Pauropoda develops exsertile vesicles in this manner on the collum alone. The median strand, absent in the Pauropoda, is incorporated in the Symphala and Diplopoda into the ventral nerve cord. The paired gonoducts arise as ventral invaginations of ectoderm in a progoneate position, on the tenth serial segment in the Symphala but on the eighth serial segment in the Pauropoda and Diplopoda. The salivary glands in these three myriapod classes are maxillary and mesodermal though the tentorial arms, apodemes and tracheae are as in chilopods. The dorsal extra embryonic ectoderm develops a temporary dorsal organ.

Taking the entire course of embryonic development into account, there is evidence at every level, from cleavage through presumptive area formation to the further development of the midgut, mesoderm and ectoderm, that the Chilopoda have arisen from one line of myriapod evolution and the Symphyla, Pauropoda and Diplopoda from another. The same evidence further suggests that the Pauropoda and Diplopoda are more closely related to one another than either is to the Symphyla but does not give a clear indication of the relative timing of their divergences from a common ancestry. A possible solution to this problem emerges from a reconsideration of the comparative development of the myriapod head. All myriapods share the same basic construction of the head as far back as the maxillary segment, but differ in the functional expression of the postmaxillary segment. In the Pauropoda and the Diplopoda this segment remains part of the trunk, but specialized as the limbless collum. In the Symphyla as in the Chilopoda, the postmaxillary segment is incorporated into the head, but retains limbs modified as jaws.

3. CRUSTACEA

The Crustacea, a class which shows great variety in modes of living and morphological structure of its members, is also diversified with respect to their developmental processes. However, these animals have some noteworthy characteristics in common. Basic crustacean development originates from a small egg, passes through a total cleavage and proceeds to hatching as a small planktonic larva, the nauplius (Fig. 10.3.1). In species having ova with little yolk, this stage represents the first free-living larva, while it is passed through during the embryonic life of species with yolky eggs. The mode of cleavage of crustacean eggs retains vestiges of the spiral type found in annelids. Another characteristic feature of the crustacean development is the teloblasts also play an important role in forming the body region posterior to the first maxillary segment. After hatching, the larval stages combine an elaborate functional morphology with a continued development, interrupted by moults and culminating in a greater or lesser degree of metamorphosis, all within relatively small dimensions. The various complicated structures of all the adult Crustacea are built up in conformity with the general ontological principle

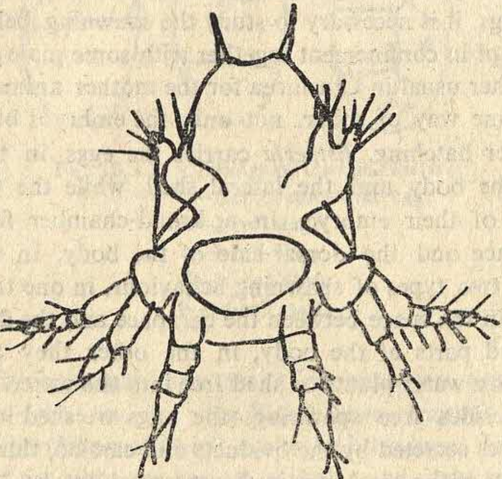


Fig. 10.3.1. Eucopepodian nauplius larva.

that their bodies are constructed on a fundamental plan consisting of a succession of segments, each of which carries a pair of biramous limbs. The appearances of a habit such as the breeding of the young exerts a definite influence on the amount of yolk in the ova and this in turn, causes variations in the mode of embryonic development as well as the time of hatching. The habit of caring for their offspring is highly developed among the Crustacea. In the majority of cases, the young are carried on the bodies of the mother animals, and can easily be obtained by collecting such brooding adults. To secure uncleaved eggs, it is necessary to study the spawning behaviour of females kept in confinement together with some male animals.

It is rather usual in Crustacea for the mother animal to protect in some way or other, not only the embryos but also the larvae after hatching. *Estheria* carries the eggs in the spaces between the body and the lateral shell, while the Cladocera take care of their embryos in a brood-chamber formed by the carapace and the dorsal side of the body. In Ostracoda there are two types of spawning behaviour, in one the ova are protected in the space between the carapace and the dorsal side of the hind parts of the body, in the other they are either deposited on water plants or shed free into the water. In Eucopepoda besides free spawning, the eggs are shed in numbers into egg sacs secreted by the oviducts and carried, thus attached to the body of the mother animal until they hatch. Among the Malacostraca, the Peracarida form a brood chamber under the thorax by the imbrication of foliaceous oostegites which develop from the bases of the maternal pereiopods. Except for *Penaeus* and allied species, which shed their eggs freely into the water, all the members of the Decapoda carry their offspring attached to their pleopods. Hand-in-hand with the development of the breeding habit there generally goes a retardation in the stage at which the larva begins an independent life although the time of hatching is not always similarly affected.

The crustacean eggs are rather large and yolky. The size of an egg depends mainly on the amount of yolk it contains and bears no relation to the size of the mother animal. The eggs are centrolecithal, irrespective of their size. The protoplasmic portion of an uncleaved egg contains a thin superficial layer of peripheral protoplasm and a protoplasmic island isolated in the centre of the mass of yolk which occupies the greater part of the egg (Figs. 10.3.2 and 10.3.3). The egg axis is indicated by the location of the polar bodies. The Crustacea have one or two egg membranes.

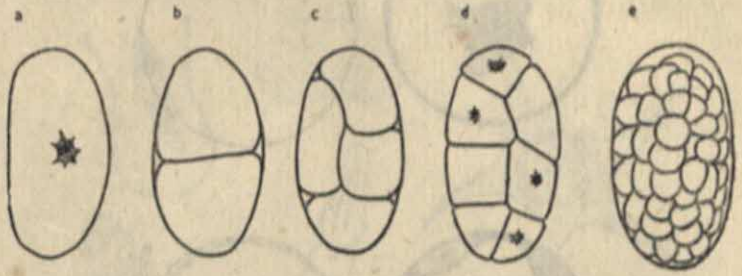


Fig. 10.3.2. Cleavage of *Caridina laevis*.
a—L.S. of uncleaved egg.
b—2-cell stage.
c—4-cell stage.
d—16-cell stage.
e—64-cell stage.

Generally speaking, crustacean spermatozoa are atypical, even such forms as are flagellated have unusual structural features. Spermatozoa which are to be introduced directly into the oviduct are simply spherical in shape and only small numbers of them are produced. When fertilization is to take place within the brood chamber, much large numbers of smaller, but more complex spermatozoa are formed. Various types of spermatozoa found among the orders of Crustacea are given in Fig. 10.3.4. The spermatozoa of the Malacostraca and Copepoda are passed to the female packed into spermatophores, which are enclosed in a substance secreted by the terminal part of the vas deferens. The spherical, ovoid or sausage-shaped spermatophore, of a chitin-like texture, is stuck to the gonopore of the female. Besides the spermatozoa, it also contains a secretion from the vas deferens which swells within the female receptaculum seminis and causes the spermatophore covering to burst.

Our knowledge concerning fertilization in the Crustacea is rather limited, since observation is made difficult by the opacity

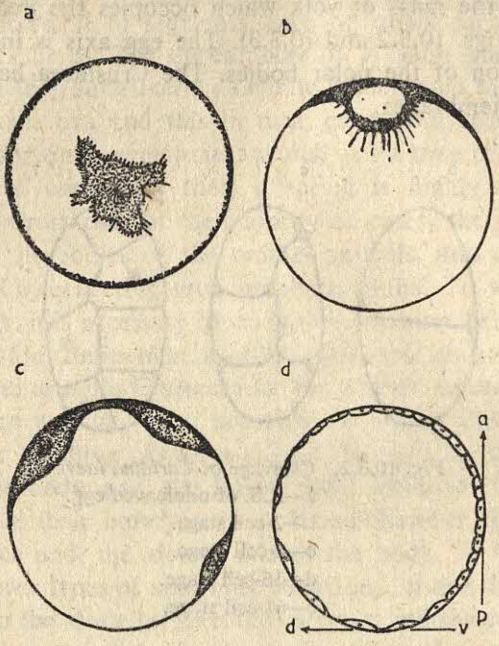


Fig. 10.3.3. Cleavage of *Nebalia bipes*.
a—Uncleaved egg immediately after spawning.
b—Beginning of first cleavage.
c—Early cleavage stage.
d—Blastula.
a—anterior; d—dorsal side;
p—posterior; v—ventral side.

of their eggs, owing to the abundance of yolk and the rare occurrence of free egg laying, as a result of the highly developed breeding habit.

Early Development

With a few exceptions on both sides, the eggs of Entomostraca, which have relatively less yolk, undergo total cleavage, whereas superficial cleavage is the rule in the yolky eggs of the Malacostraca.

Cleavage

Holoblastic cleavage: In the cirripede, *Tetraclita rosea* the long axis of the egg is always the anteroposterior axis

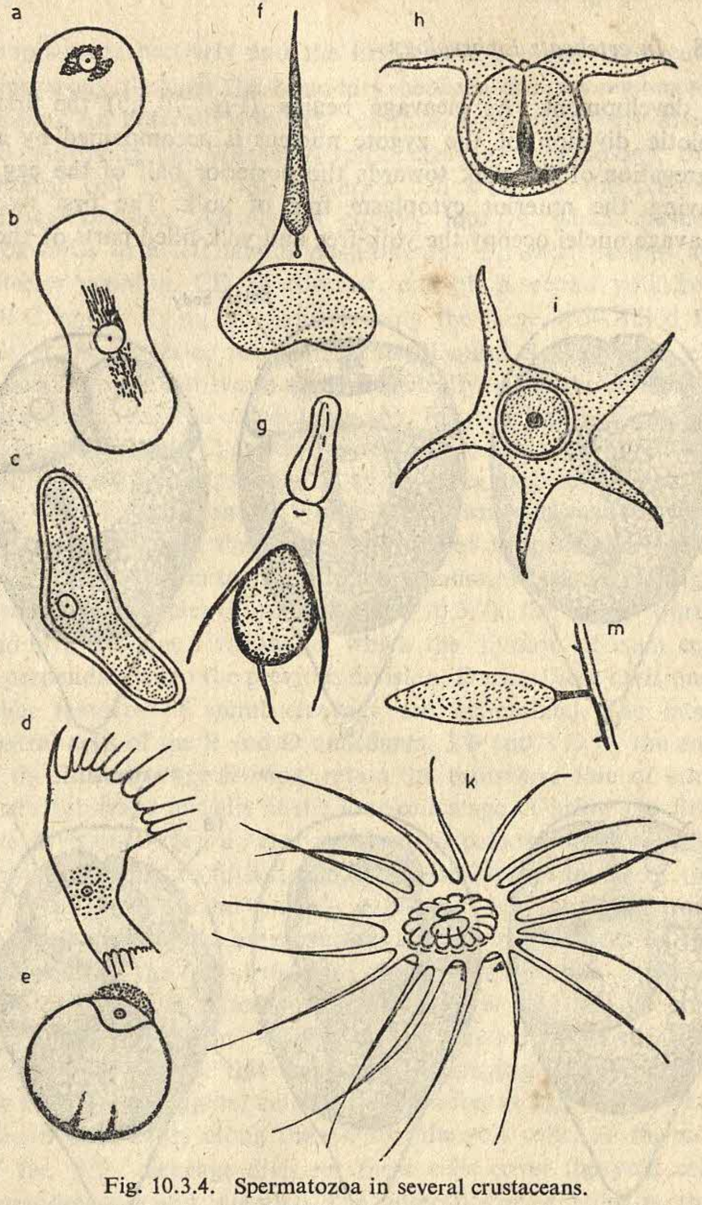


Fig. 10.3.4. Spermatozoa in several crustaceans.

a—*Latona*.
b—*Daphnella*.
c—*Moina*.
d—*Polyphemus*.
e—*Squilla*.
f—*Leander*.
g—*Galathea*.
h—*Ethusa* (lateral view).
i—*Ethusa* (upper side).
k—*Astacus*.
m—Spermatophora of *Galathea*.

of development. As cleavage begins (Fig. 10.3.5) the first meiotic division of the zygote nucleus is accompanied by a segregation of the yolk towards the posterior half of the egg, leaving the anterior cytoplasm free of yolk. The first two cleavage nuclei occupy the yolk-free and yolk-filled parts of the

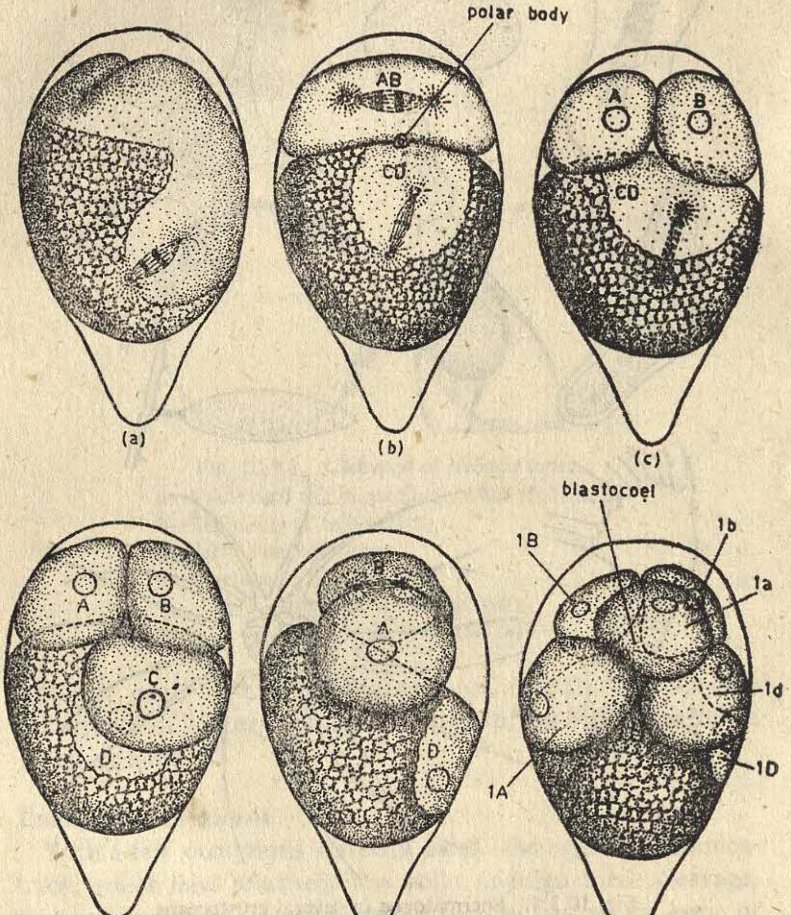


Fig. 10.3.5. Early cleavage in the cirripede, *Tetraclita rosea*.
A—First cleavage division.
B to D—Second cleavage division sequence, dorsal view.
E—4-cell stage (left lateral view).
F—8-cell stage (left lateral view).

cytoplasm respectively and the first cleavage plane then cuts transversely through the boundary between the two regions to separate an anterior yolk-free cell from a posterior, yolk-filled cell. Adopting the Wilsonian spiral cleavage terminology, the anterior cell is AB, the posterior cell CD. At the second cleavage division, AB divides equally into A, which pushes backwards to a left lateral position, and B, which retains an anterior position. CD, in contrast, cuts off a second, yolk-free cell C, anteriorly on the right, leaving the large, yolk-filled D cell in the posterior position. As in all spiral cleavage, the cell B and D retain transverse contact ventrally, while the A and C cell establish sagittal contact dorsally. In typical spiral cleavage, D is dorsal, and B is ventral relative to the axes of future development, but the shift of B to an anterior position and D to a posterior position is a typical modification associated with increased yolk and the entire sequence of cirripede cleavage is modified in relation to yolk. All four quadrants proceed through three division cycles (Figs. 10.3.6 and 10.3.7), the third, fourth and fifth cleavage divisions, in which the division of each cell is perpendicular to the previous division. During these divisions, other features of spiral cleavage are maintained. The most ventral cells of the B and D quardants, 3 B and 3 D by the end of the fifth cleavage division, retain the transverse line of contact established initially at the four-cell stage. During the first five division cycles, no cleavage furrows pass through the yolk. During the third to fifth cleavages, the division products of the yolk-free B-cell spread ventrally backwards over the D-quadrant yolk cell to cover the anteroventral and ventral surfaces of the yolk cell. At the end of the fifth cleavage division the daughter cells of B are, in anteroposterior order, $1b^l$ and $1b^r$, $2b^l$ and $2b^r$, 3b and 3B. The 1b cells do not participate in the fifth cleavage division at this stage. Accompanying the divisions of the B cell, the daughter cells of the yolk-free A and C cells also spread backwards along the sides of the yolk cells. At the end of the fifth cleavage division, these cells cover the yolk cell anterodorsally and laterally. The anteroposterior order is the left $1a^a$, then $1a^{pd}$, $1a^{pv}$, $2a^d$ and $2a^v$ in a transverse row and 3a and 3A behind. 3A makes contact with the left side of the mid-ventral cells 3B. The C quadrant cells on the right are in the same order, $1c^a$, then $1c^{pd}$, $1c^{pv}$, $2c^d$ and $2c^v$ in a transverse

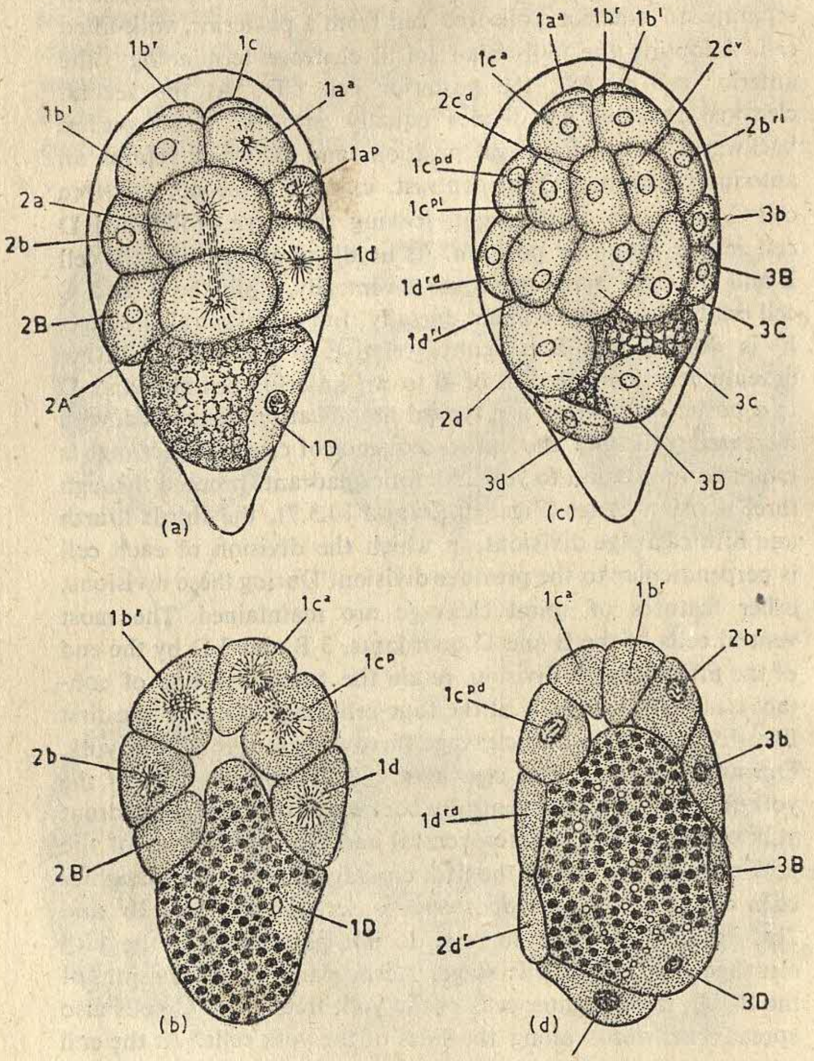


Fig. 10.3.6. Late cleavage in the cirripede *Tetraclita rosea.*
A—Fourth cleavage division (left lateral view).
B—Sagittal section through the stage shown in 'A'.
C—28-cell stage (right to lateral view).
D—Sagittal section, through the stage shown in 'A'.

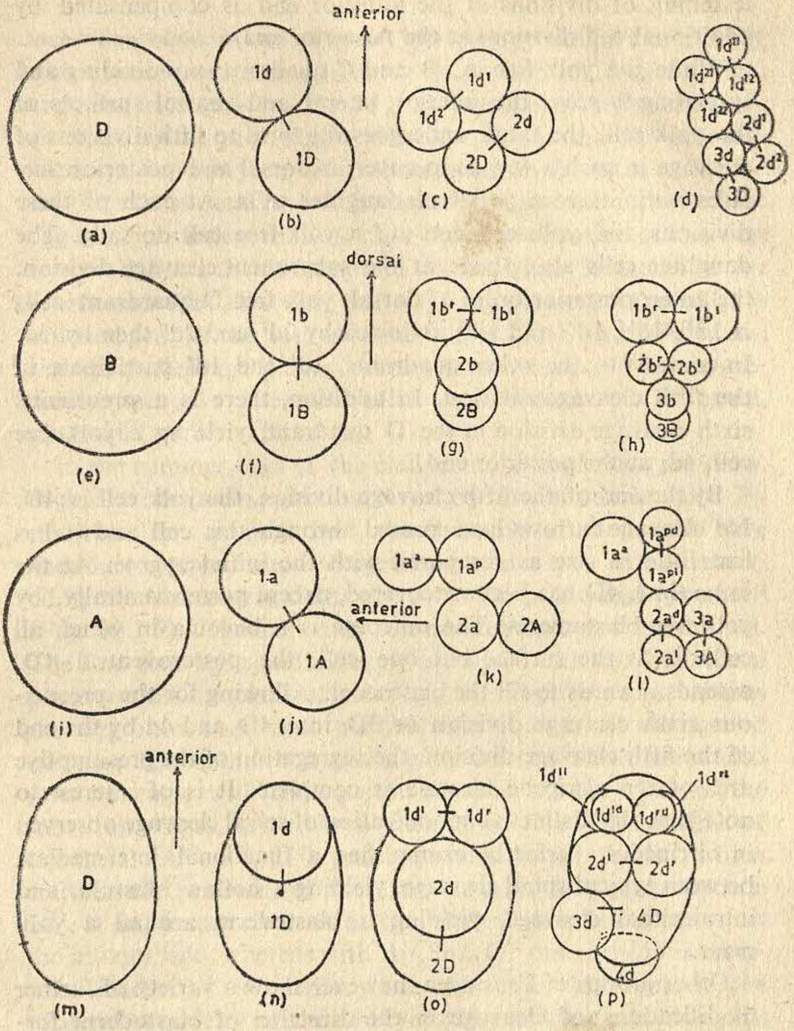


Fig. 10.3.7. The modifications of spiral cleavage in the four quadrants of the cirripede egg.

row, and 3c and 3C behind. The latter is, of course, in contact with the right side of 3B. It will be noticed that, as in the B quadrant, the most anterior cells of the A and C quadrants ($1a^a$ and $1c^a$) do not participate in the fifth cleavage division. This lessening of divisions at the anterior end is compensated by additional cell divisions at the posterior end.

While the yolk-free A, B and C quadrants are dividing and spreading to cover the anterior, lateral and ventral surfaces of the yolk-cell, the latter undergoes the third to fifth divisions of cleavage in such a way as to cover its dorsal and posterior surfaces with its own yolk-free daughter cells. At each of these divisions the yolk cell cuts off a yolk-free cell dorsally. The daughter cells also divide at the subsequent cleavage division, the anteroposterior order of dorsal, yolk-free D-quardrant cells is $1d^{ll}$, $1d^{ld}$, $1d^{rd}$ and $1d^{rl}$, followed by $2d^{l}$ and $2d^{r}$ then by 3d. In contrast to the other quadrants, $1d^{l}$ and $1d^{r}$ participate in the fifth cleavage division. In addition, there is a precocious sixth cleavage division in the D quadrant, yielding a yolk-free cell, 4d, at the posterior end.

By the end of the fifth cleavage division, the yolk cell is 4D. No cleavage furrows have passed through this cell and it has lost little in size as compared with the initial zygote. At the same time, 4D has become covered, except posteroventrally, by yolk-free blastomeres. The outcome is a blastula in which all cells lie at the surface but one cell, the posteroventral 4D, extends inwards to fill the blastocoel. Allowing for the precocious sixth cleavage division of 3D into 4D and 4d by the end of the fifth cleavage division, the segregation of the presumptive area of the cirripede blastula is complete. It is of interest to note that the distinctive modification of spiral cleavage observed in cirripedes, perfectly exemplifies a functional intermediate between typical spiral cleavage, yielding a hollow blastula, and intralecithal cleavage, yielding a blastoderm around a yolk mass.

Other groups of Crustacea, however show a variety of further modifications of cleavage in the direction of blastoderm formation. Among the Branchiopoda, the cladoceran *Holopedium* with a spherical egg 120μ in diameter, retains a sequence of mitotic nuclear divisions whose spiral pattern is even more distinct than that of the cirripedes but has a much more specialized sequences of cytokineses associated with them. No cleavage furrows penetrate the cytoplasm of the *Holopedium* egg until after the third synchronous nuclear division has taken place and the eight resulting nuclei are approaching the egg surface. The eight nuclei (Fig 10.3.8) occupy octants of the egg which subsequent events identify as dorsal, left, anterior right and posterior (the 1a, 1b, 1c and 1d octants) and ventral left, anterior, right and posterior (the 1A, 1B, 1C and 1D octants). Cleavage furrows then cut in simultaneously from the surface to separate these octants, but the densely yolky central part of the egg remains uncleaved. A similar radial penetration of cleavage furrows follows the formation of 16 and then 31 nuclei at the fourth and fifth cleavage, but total separation of the cells, is attained only at the last of these stages. Thus in *Holopedium*, the early cleavage planes penetrate the yolky cytoplasm gradually and give rise eventually to a blastula of 31 equal, pyramidal, yolky cells.

In the summer eggs of the cladoceran *Polyphemus pediculus*, each ovarian ovum is accompanied by three nutritive cells. By the time the ovum is laid, the remnants of these cells have been incorporated into its vegetal part, later they will be received by the blastoderm destined to be the primordial germ cell (Fig. 10.3.9). The first and second cleavages are meridional. The first cleavage spindle lies at an angle with the equatorial plane so that the plane of cleavage, missing the poles, divides the egg into somewhat unequal blastomeres. At the second cleavage the spindles appearing in the two blastomeres also form an angle with the equatorial plane. At the close of the four cell stages, the embryo consists of three blastomeres of equal size (A^{II}, B^{II}, C^{II}), and the somewhat smaller D^{II}, which contains the remnants of the nutritive cells. B^{II}, lying opposite D^{II}, is in contact with it at the vegetal side, but separate at the animal side, whereas with A^{II} and C^{II} this relation is reversed. Since D^{II} is smaller than the other blastomeres, the bilateral symmetry of the embryo is already determined at this stage, the line connecting the centres of B^{II} and D^{II} corresponding to the future median plane, and thus indicating the antero-posterior axis. The third division occurs on a horizontal plane lying nearer the vegetal pole, and results in the formation of four larger blastomeres (a^{III}—d^{III}) in the animal hemisphere and four smaller vegetal cells (A^{III}—D^{III}). All the descendants of a^{III}—d^{III} give rise to ectoderm and are called ***primary ectoblasts*** to distinguish them from the ectoderm cells which will be derived from A^{III}—C^{III}. These blastomeres, termed

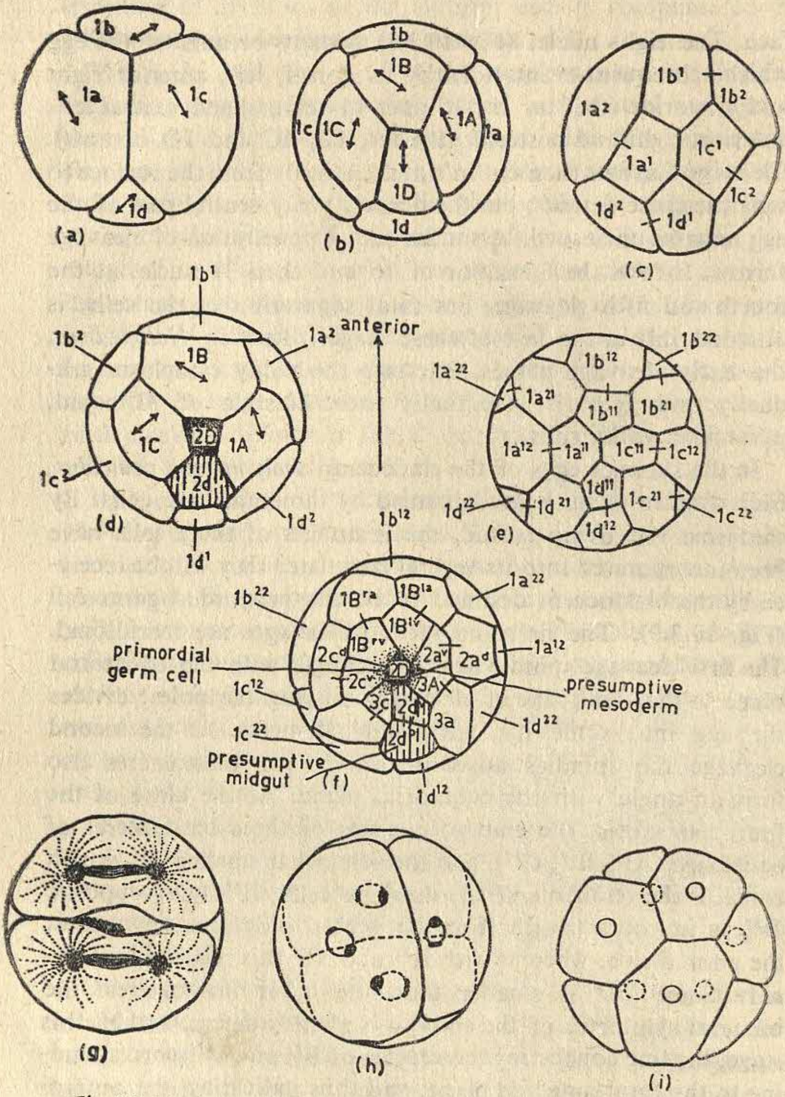


Fig. 10.3.8. a-f. 3rd to 5th cleavage divisions of the cladoceran *Holopedium*.
a—8-cell stage (dorsal view).
b—8-cell stage (ventral view).
c—16-cell stage (dorsal view).
d—13 to 16-cell stage (ventral view).
e—31-cell stage (dorsal view).
f—31-cell stage (ventral view).
g-i—Early cleavage in the anostracan, *Artemia salina*.

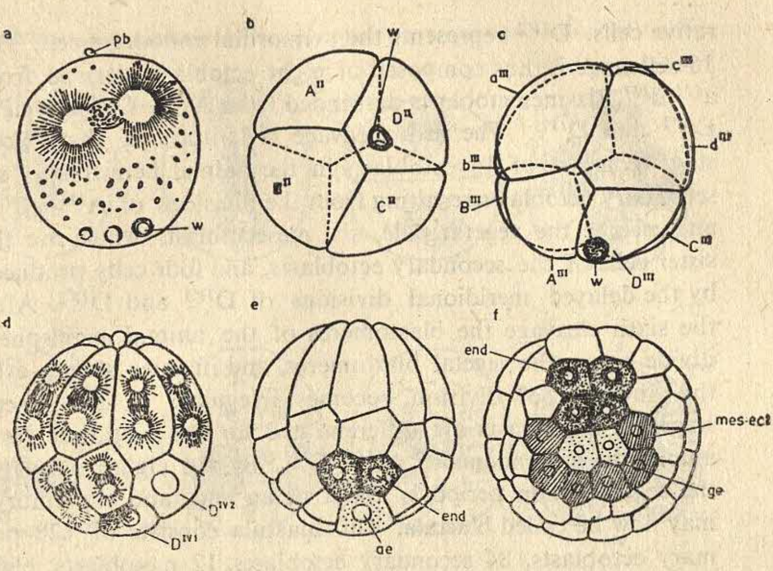


Fig. 10.3.9. Cleavage in *Polyphemus*.
a—First cleavage (cross section).
b—4-cell stage (from ventral pole).
c—8-cell stage (lateral view).
d—16 to 32-cell stage (lateral view).
e—31-cell stage (frontal view).
f—62-cell stage (from vegetal pole)·
A^{III}—D^{III}—blastomeres in vegetal half.
a^{III}—d^{III}—blastomeres in animal half; end—endoblast; ge—primordial germ cell; mes.ect—mesectoblast; pb—polar body; w—remnant of nurse cell.

mesectoblasts, later form ectoderm and mesoderm, whereas D^{III} develops into endoderm and the primordial germ cell. Although cleavage is partial in the earlier stages, the cleavage furrows grow deeper and deeper as development proceeds; at the close of the third cleavage all the blastomeres are completely separated from each other for the first time and small segmentation cavity is formed at their centre. The fourth cleavage is meridional in all the blastomeres except D^{III}. D^{III} divides later than the others and latitudinally into D^{IV1} and D^{IV2}, the former lying nearer the vegetal pole. D^{IV1} which includes all the remnants of the nutritive cells, is now differentiated into the primordial germ cell, and all its descendants give rise to generative cells. D^{IV2} represents the primordial endoderm cell. The 16-cell stage is thus composed of eight ectoblasts derived from a^{III} d^{III}, six mesectoblasts descended from A^{III}—C^{III} as well as D^{IV1} and D^{IV2}. The fifth cleavage is latitudinal; the 32-cell stage consists of 16 ectoblasts in the animal hemisphere, six secondary ectoblasts resulting from the divisions of A^{III}—C^{III}, and nearer the vegetal pole, six mesectoblasts which are the sister cells of the secondary ectoblasts, and four cells produced by the delayed meridional divisions of D^{IV1} and D^{IV2}. After the sixth cleavage the blastomeres of the animal hemisphere divide before the vegetal blastomeres, and in later stages, even the direction of division becomes irregular. In the 48-cell stage, six mesoblasts are differentiated for the first time from among the descendants of A^{III}—C^{III}. By the eighth cleavage, the segmentation period is about at an end, and the embryo may now be called blastula. This blastula consists of 128 primary ectoblasts, 84 secondary ectoblasts, 12 mesoblasts, eight endoblasts and four generative cells—a total of 236 cells enclosing a relatively spacious blastocoel. The mesoblasts surround the primordial germ cells in a U-shaped formation, and the entoblasts lie between the germ cells and the equator. Eight ectoblasts situated at the animal pole are larger than the others; these give rise to the anlage of the *apical plate*, which will form the brain. Gastrulation which begins about the time of the ninth cleavage is effected by a sinking of the mesoblasts, endoblasts and germ cells in the blastocoel, rather than by a clear-cut invagination.

The egg of *Cyclops viridis* undergoes total and equal cleavage from the outset. At the time of first cleavage, granules known as ectosomes, crowd around one of the asters; in succeeding divisions these will always enter only one of the blastomeres. In the fifth cleavage the blastomere containing the ectosomes divides into a primordial germ cell, which receives all the granules, and a primordial endogerm cell (Fig. 10.3.10). When the seven blastomeres succeeding these cells undergo the sixth cleavage, they give rise to the mesoblasts. After the eighth cleavage, the four endoderm cells and two germ cells, together with the mesoderm cells, move into the blastocoel where they form a plug like mass. The blastopore invagination which

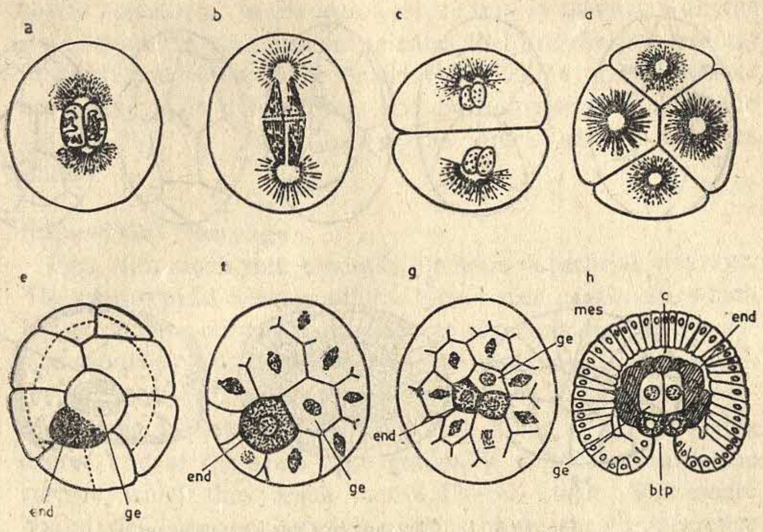


Fig. 10.3.10. Cleavage in *Cyclops viridis*.
a—Uncleaved egg.
b—Just division.
c—2-cell stage.
d—4-cell stage.
e—16 to 32-cell.
f—Sixth division.
g—Seventh division.
h—c.s. of gastrula.
blp—blastopore; e—segmentation cavity; end—endoblast; ge—primordial germ cell; mes—mesoblast.

results from their sinking inward is closed after the end of the ninth cleavage.

The egg of *Cypris incongruens* is oblong, ovate, with its primary axis vertical to the long dimension (Fig. 10.3.11). Its cleavage is total and equal from the beginning. There are neither size differences among the blastomeres, nor such peculiar granules as are found in the *Cyclops* eggs. The traits of spiral cleavage are more conspicuous in this species than in the two examples cited above. From the third cleavage onwards, cell divisions tends to begin first in the blastomeres at the animal pole, and successively later towards the vegetal pole. This time-lag in division according to the location of the blastomeres be-

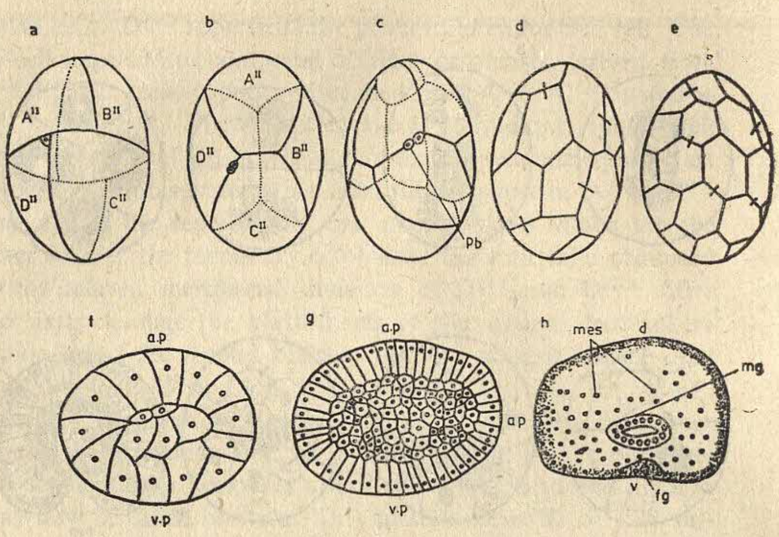


Fig. 10.3.11. Cleavage in *Cypris incongruens.*

a—4-cell stage, early cleavage furrows.
b—Late 4-cell stage.
c—8-cell stage.
d—16-cell stage.
e—32-cell stage.
f—134-cell stage.
g—Blastula.
h—Gastrula.

ap—apical plate; a.p.—animal pole; d—dorsal side of embryo; fg—foregut; mes—mesoderm; mg—midgut; pb—polar body; v—ventral side of embryo; v.p.—vegetal pole.

comes increasingly marked as development progresses, until finally in the eighth cleavage cycle, division begins first at the vegetal pole and proceed towards the animal pole. At this time, five to eight endoderm cells are constricted into the interior of the embryo, which is thus transformed into a morula-like blastula. The embryo subsequently undergoes an increase in the number of cells making up its outer layer as well as its inner mass and during a long resting stage, the endoderm cells, which have lost their cellular boundaries accumulate at the ventral side while the yolk becomes concentrated at the dorsal side. The endoderm cells then differentiate into a small sac-like midgut (mesenteron) anlage, and the remaining inner cells becomes mesoderm. In the region where it is in contact with the mesenteron the ectoderm invaginates to form the primordium of the stomodaeum; at the anterior end of the ventral surface an apical plate differentiates and posterior to this along the median line the primordium of the ventral nerve cord takes shape.

Superficial Cleavage

Eggs with much yolk generally undergo superficial cleavage. The most typical cleavage of this type is seen in *Astacus*, which has extremely yolky eggs. Its cleavage consists simply in successive subdivision of the nucleus and protoplasmic island, which do not involve the yolk mass as a whole. As development proceeds, the nuclei separate from each other to an increasing degree and at the same time gradually migrate towards the surface, which they reach in the 128-cell stage. Blastomere boundaries, surrounding each nucleus, make their appearance abruptly and synchronously over the whole surface in the 512-cell stage, and under the influence of these cells, the yolk mass is simultaneously divided into a corresponding number of elongate pyramidal masses called primary yolk pyramids, the apices of which converge toward the centre of the egg (Fig. 10.3.12). After the 128-cell stage, the nuclear divisions on one side of the embryo lag behind those on the other side. Gastrulation begins at the 102-cell stage.

In the egg of *Squilla oratoria*, all the cleavage nuclei reach the peripheral cytoplasm by the 128-cell stage and the cleavage furrows appear toward the end of this stage. The yolk pyramids, however are very short in this species, the furrows extending only a short distance into the yolk and advancing very little further even in the succeeding stages, so that the central yolk remains homogeneous. At this stage with about 500 cells, regional differences in the distribution of nuclei almost obliterate the yolk pyramids, although the cell boundaries remain visible on the surface.

In the egg of *Panulirus japonicus*, the cleavage furrows are abruptly and simultaneously established in the eight-cell stage (Fig. 10.3.13). When the eight-celled embryo cleaves, the right and left sides of the new furrows meet the earlier ones at sepa-

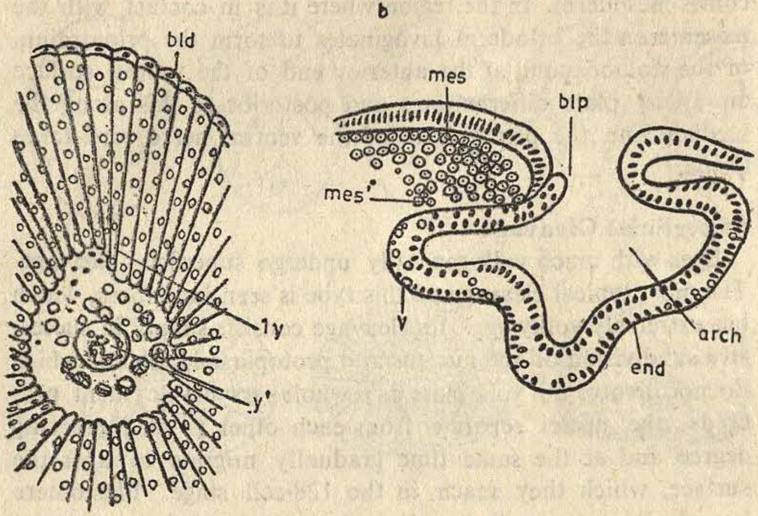


Fig. 10.3.12. Embryo of *Astacus fluviatilus.*
a—c.s. through part of blastula.
b—l.s. through blastopore of gastrula.
ly—primary yolk pyramid; arch—inner cavity of archen-teron; bld—blastoderm; blp—blastopore; end—endoderm; mies—mesoderm; mes—degenerated nuclei of secondary mesoderm; y—yolk granule; y—central yolk body.

rate points. Subsequent shifting of the blastomeres into a more stable arrangement changes the directions of the furrows so that they form continuous zig-zag lines. This behaviour can definitely be regarded as a vestige of spiral cleavage. In the 18-cell stage, the furrows penetrate deeply into the yolk, dividing this into complete blastomeres with distinct inner as well as superficial boundaries. The major part of the yolk is distributed among these blastomeres, but since the furrows do not reach the very centre of the egg, a small amount of yolk which has not been included with the cells remains there. In other words the space corresponding to blastocoel is filled with yolk. As the nuclei continue to divide and move out to the surface, the height of the blastomeres gradually decreases enlarging the blastocoel; by the 128-cell stage all the nuclei have reached the surface. The outer surface of each blastomere is covered by a

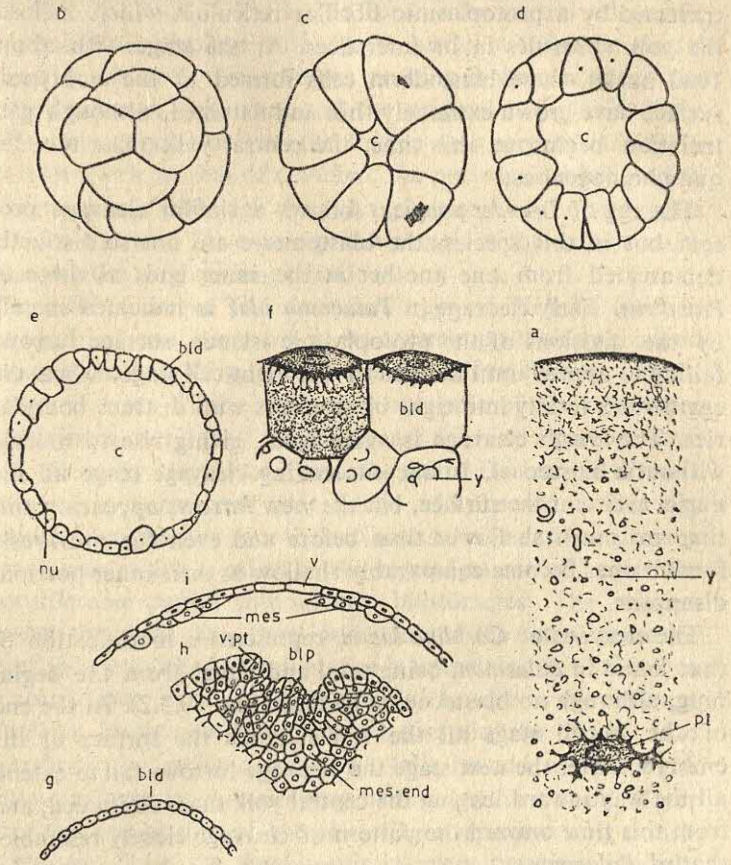


Fig. 10.3.13. Cleavage and formation of germ layer in *Panulirus japonicus*.

a—c.s. of part of egg at 2-cell stage.
b—Surface view of 16-cell stage.
c—c.s. of same.
d—c.s. of 64-cell stage.
e—c.s. of 350-cell blastula.
f—Enlarged sketch of blastoderm cells of same blastula.
g—c.s. of part of blastula.
h—c.s. of part of gastrula.
i—c.s. through blastoderm of gastrula.

bld—blastodermal cell; blp—blastopore; c—segmentation cavity filled with yolk; mes—nauplius mesoderm; mesend—meso-endoderm cell complex; nu—regressing nucleus; pl—protoplasmic island; b—ventral side; v.pt.—ventral plate rudiment; y—yolk.

protoplasmic layer containing the nucleus, and its interior is traversed by a protoplasmic fibrillar reticulum which encloses the yolk spherules in its interstices. At the stage with about 1000 nuclei, these blastoderm cells formed at the embryonic surface have grown extremely thin and flattened, although gastrulation begins at this time, the central yolk mass remains quite homogenous.

The egg of *Leander pacificus* follows a similar cleavage process, but in this species, the blastomeres are not so distinctly demarcated from one another at the inner ends as those of *Panulirus*. Early cleavage in *Palaemon idae* is indicated merely by the divisions of the protoplasmic islands, surface furrows failing to appear until the end of the eight-cell stage, when the egg divides totally into eight blastomeres with distinct boundaries. The fourth cleavage is again total, giving rise to morula without a blastocoel. In the succeeding cleavage stage all the nuclei arrive at the surface, but the new furrows appearing this time are much shallower than before and even the previously formed ones become comparably shallow as their inner portions disappear.

The cleavage of *Caridina laevis*, represents a modification of that found in *Palaemon*, being total and equal from the beginning, although no blastocoel is formed (Fig. 10.3.2). At the end of the 16-cell stage all the nuclei reach the surface of the embryo. After the next stage the cleavage furrows fail to extend all the way inward leaving the central yolk mass undivided, and from this time onward the pattern of cleavage closely resembles that of *Palaemon*.

In the Malacostraca generally, it is rather difficult to follow the polar axis definitely from the uncleaved egg to the stage of blastoderm formation because the polar bodies are absorbed sooner or later into the embryo. Further, there is no perceptible variation among the exposed surfaces of the blastomeres to use as an indicator of the egg axis. This situation changes, however, with blastoderm formation. As will be described later, a greater concentration of nuclei in one hemisphere of the embryo induces the formation of the germinal disc, and the blastopore invagination at one point on the periphery of the disc defines the orientation of the antero-posterior and dorsoventral axes. The site of blastopore corresponds to the posterior end, and the line between it and the opposite side of the disc represents the antero-posterior axis. The disc itself is destined to become the ventral side, while the opposite hemisphere with sparse nuclei will be the dorsal side (Fig. 10.3.14). Nevertheless nothing is known about the relation between these axes and the original polar axis. Since the first cleavage plane cuts the longer axis perpendicularly in oval eggs such as that of *Caridina*, the polar axis is believed to lie in this plane, although the exact positions of the two poles remain unknown. The antero-posterior axis of the embryo coincides with the original longer axis.

Discoidal cleavage: The uncleaved eggs of *Nebalia bipes* is centrolecithal and possesses at its centre a protoplasmic island containing nucleus (Fig. 10.3.3). Before the first cleavage begins, the island rises to the surface at one pole of the egg, there undergoing a horizontal cleavage and forming a protoplasmic mass distinctly separated from the yolk. Horizontal division continues in subsequent stages, the cells formed in this way spreading peripherally over the surface as their number increases. A small area which is called as the vegetal pole is left behind for a considerable period without any blastomeres. The relation between the polar axis thus indicated and the antero-posterior axis of the established germinal disc is uncertain. In addition to this normal process, various abnormalities occur in the cleavage of *Nebalia*. Sometimes the germinal disc may be divided into a number of disc-like groups of cells instead of forming a continuous layer, or the cells may be arranged in a double layer before the completion of the disc. These represent only transient phases, since the final result in every case is a blastula enclosed in a single-layered blastoderm, as in other malacostracans.

Germ-layer Formation

Since gastrulation and germ layer differentiation in the holoblastic eggs have already been dealt with, the following description will be confined to these processes as observed in the eggs which undergo superficial cleavage, as shown by the embryo of *Panulirus japonicus*. As soon as the blastoderm is established, its constituent cells begin to accumulate in the region destined to be the future ventral side, so that the surface of the embryo becomes divided into two hemispheres, one densely and the

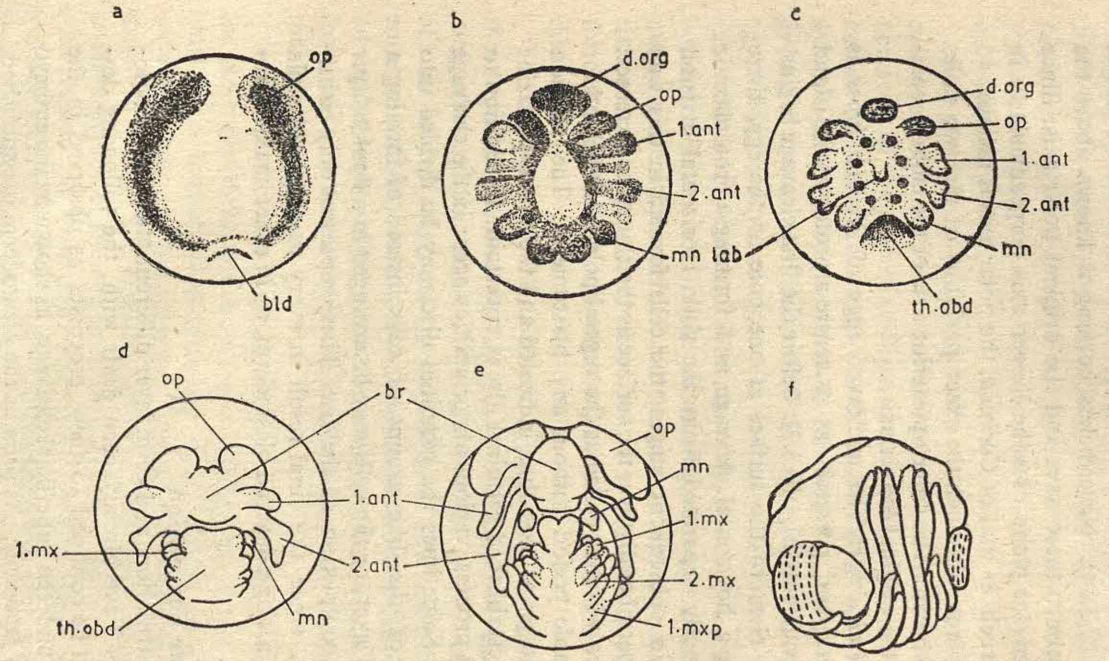


Fig. 10.3.14. External appearance of *Panulirus japonicus* embryo.

a—Embryo at formation of blastoderm.
b—Pronauplius stage.
c—Nauplius stage.
d—Stage with 7 pairs of appendages.
e—Stage with 11 pairs of appendage.
f—Embryo shortly before hatching (frontal view).

1 ant—first antenna; 1 m—first maxilla; 1 mxp—first maxilliped; 2 ant—second antenna; e mx—second maxilla; br—brain; blp—blastopore; d.org—dorsal organ; lab—labrum; min—mandible; op—optic lobe; th.abd—thoracic abdominal process.

other sparsely nucleated. The densely nucleated hemisphere, which represents the primordium of the germinal disc, forms at one point on its periphery a small area particularly crowded with nuclei. This area sinks to form a shallow invagination, from which the cell actively migrate into the interior (Fig. 10.3.13). This constitutes the blastopore, which lies at the posterior end of the embryo (Fig. 10.3.14). Within a short time, a horse-shoe shaped accumulation of cells appears on the germinal disc, extending anteriorly from the blastopore. Each of the free ends is a mass of especially tall cells; these form two small areas called the *optic lobes*, which will develop later into the compound eyes. The appendages and their respective ganglia will differentiate on the rest of the band. Out of the cells migrating inward from the blastopore, some form a pair of cellular bands proceeding forward along the underside of the horseshoe while others accumulate in a plug-like mass beneath the blastopore. The former represents the naupliar mesoderm and the latter, the so-called undifferentiated mesentoderm cell-complex. These cells gradually enlarge as they take in yolk granules from the surrounding yolk mass. During the process, the amount of their cytoplasm fails to increase fast enough to keep pace with such rapid enlargement and is stretched into a thick layer, like a membrane. After this stage, the blastopore closes and on either side of it the rudiments of the ventral plate, which gives rise to the posterior body segments, make their appearance as a pair of ectodermal elevations which very soon unite. The vestige of the closed blastopore takes a position on the ventral plate and becomes the anal rudiment.

About this stage, on the two arms of the horse-shoe shaped ectodermal band in the region between the blastopore and the optic lobes, three pairs of limb rudiments are raised from the surface as transverse ridges. These acquire distinct shape in the sequence; mandibles, antennules and antenna. In front of the optic lobes on the median line the dorsal organ is formed as an assemblage of tall ectodermal cells. By inward development of the ectodermal folds, these limb rudiments grow into tubular processes projecting over the germinal disc and the naupliar mesoderm extends into their interior to constitute the limb mesoderm. Near the bases of these limbs and optic lobes, the ganglion primordia are formed as ectodermal thickenings arranged in pairs on either side of the median line. The stomodaeum invaginates in the region between the antennules and the antenna and its anterior wall grows backward over the oral aperture to cover it as a labrum. Since the stage with these three pairs of appendages is equivalent to the free-living nauplius larva, it is called the *egg-nauplius*. The germinal disc of this embryo corresponds to the ventral surface of the nauplius.

By the time the limbs assume their several shapes, the development of the so-called metanaupliar segments (maxillary segment and all those posterior to it) has already begun in the region posterior to the limb rudiments. The constituent elements of the mesendoderm complex which has pushed its way into the yolk from the blastopore region as a plug-like mass, absorb yolk from their surroundings and gradually change into vesicular cells, which develop as a whole into a sac like mass called the yolk sac (Fig. 10.3.15). The sac remains connected with the blastopore even after the latter has been converted into the proctodaeum, through a slender neck. Its constituent cells, which represent the endoderm, continue to grow gradually into elongate pyramids and finally take the whole yolk mass. These cells called as the secondary yolk pyramids have very thin protoplasmic membranes and their nuclei are situated on the periphery of the yolk mass as in the primary yolk pyramids (Fig. 10.3.16). The yolk sac represents mesenteron which will later be replaced by epithelial endoderm.

Before this, in an earlier stage of yolk sac development, two characteristic cells appear on either side of the neck of the sac within the still undifferentiated sub-blastoporic mesendoderm cell complex. No yolk granules are included in their cytoplasm, they have large, strongly staining vesicular nuclei and lie close beneath the ectoderm of the ventral plate. These are the *mesoteloblasts*, which produce the mesoderm of the *metanaupliar* segments, their number will later increase to four on each side (Fig. 10.3.15). The ectoderm cells which lie directly over the mesoteloblasts subsequently differentiate into the ectoteloblasts; these closely resemble the mesoteloblasts and form a transverse semicircular line along the anterior border of the ventral plate. In the succeeding divisions, groups of both ecto- and meso-teloblasts send out daughter cells in an anterior direction and these

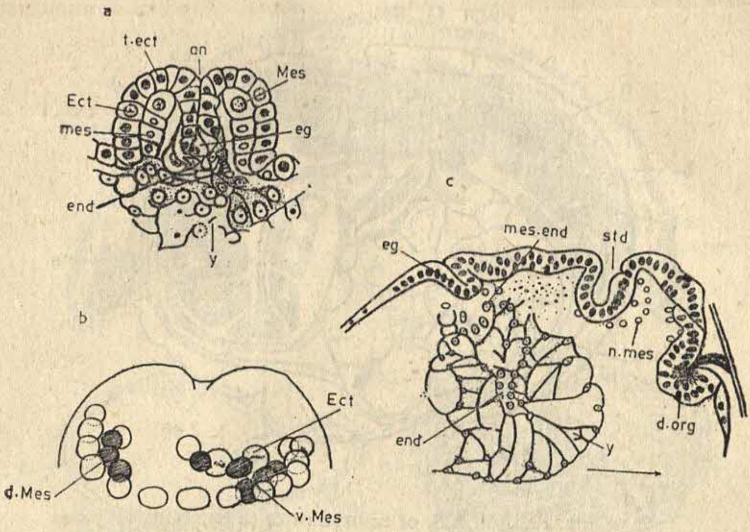


Fig. 10.3.15. Germ layer formation in *Panulirus japonicus*.
a—Nauplius larva, c.s. of ventral plate through hindgut.
b—Metanauplius stage, sketch of part of thoracic-abdominal process showing only toloblasts.
c—L.S. of larva at nauplius stage.
an—anus; d.Mes.—dorsal mesoderm cell; d.org—dorsal organ; Ect—ectoteloblast; eg—hind gut; end—endoderm; Mes—mesoteloblast; mus—daughter cell of mesoteloblast; mes.end—mes-endoderm cell complex; n.mes—nauplius mesoderm; std—stomodaeum; tect—tail ectoderm; v—Mes-ventral meso-teloblast; y—yolk.

in turn at once begin to proliferate to give rise to the maxillary and more posterior segments. By such addition of new segments and their growth, the ventral plate develops into the thoracico-abdominal process, which arises from the posterior edge of the germinal disc and gradually elongate forward over its surface (Fig. 10.3.14). As a result, the main portion of the embryo after this stage corresponds to the anterior part of the head, and all the more posterior segments are formed on this process, which is folded over the germinal disc, or ventral side of the head (Figs. 10.3.16 and 10.3.17).

The growth of the thoracico-abdominal processes begins with cell proliferation at the ends of the ectoteloblast line. This

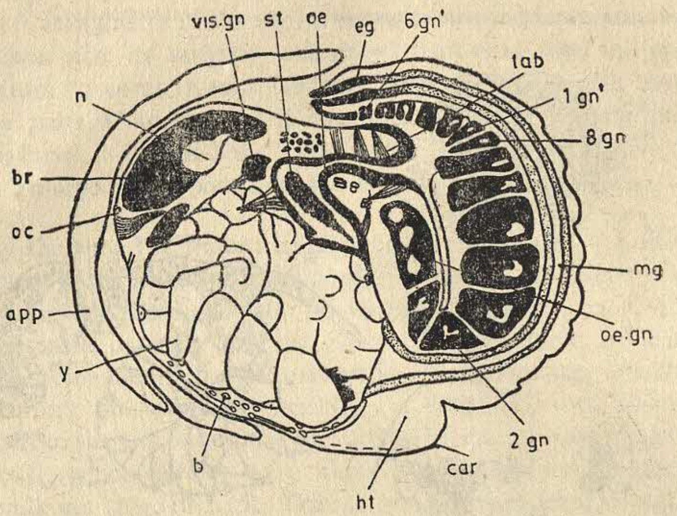


Fig. 10.3.16. L.S. of embryo prior to hatching.
1 gn′—first abdominal ganglia; 2 gn—second thoracic ganglion; 6 gn′—sixth abdominal ganglion; 8 gn—eighth thoracic ganglion; app—appendages; b—large anterior artery; br—brain; car—carapace rudiment; eg—hind gut; ht—endo-cardial cavity; lab—labrum; mg—mid gut; n—nerve fibre; oc—medean ocellus; oe—oesophagus; oe-gn—sub-oesophageal ganglion; st—stomach; vis—gen -visceral ganglion; y—yolk sac.

increase continues until 18 cells encircle the process. The ectoteloblasts themselves continue to retreat toward the tip of the process, always encircling it at its apex (Fig. 10.3.15). The anus is located on the process, always lying distal to the teloblastic ring. On each side of the process, three mesoteloblasts lie on the dorsal, and one on the ventral side of the mesenteric tube. These cells give rise to a dorsal and a ventral pair of cellular bands which extend anteriorly. Since the ring of ectoteloblasts is incomplete at first, at least a small medio-dorsal portion of a few anterior segments is formed by an extension of the dorsal head ectoderm.

Both the ecto- and meso-teloblasts lose their characteristic properties after they have finished supplying the material of the sixth abdominal segment. The telson *ectoderm* is derived from ectoderm cells occupying the area posterior to the teloblast

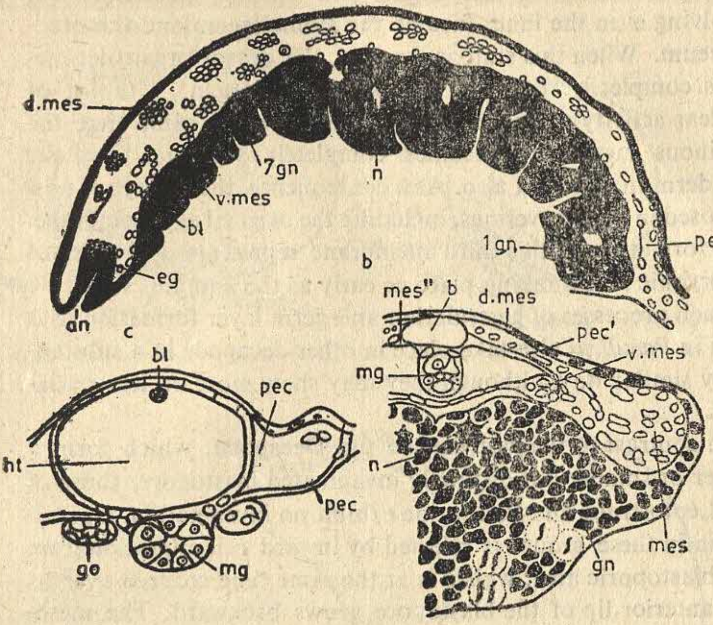


Fig. 10.3.17. Thoracico-abdominal process in *Panulirus japonicus*.
a—L.S. of thoracico-abdominal process in embryo forming compound eye.
b—C.S. of thoracico-abdominal process in embryo at same stage.
c—Formation of heart in embryo prior to hatching.
1 gn—third thoracic ganglion; i gn—seventh thoracic ganglion; an—anus; b—large posterior artery; bl—blood corpuscle; d-mes—dorsal mesoderm; eg—hindgut; gn—ganglia; go—gonad; ht—heart; mes—leg cavity mesoderm; mes″—mesoderm surrounding midgut; mg—midgut; n—nerve fiber; poc—pericardium; pec′—floor of pericardium, v—mes-ventral mesoderm.

ring. The telson mesoderm is formed by inward migration of cells from the telson ectoderm.

Another important change occurring in the egg-nauplius is the secretion of a chitinous membrane from the entire ectodermal surface, a phenomenon associated with the activity of the dorsal organ. In the cells of this organ an active nuclear metabolism and the secretion of a substance from the outer surface takes place (Fig. 10.3.15). The separation of the chitinous membrane starts at this organ and extends posteriorward, involving even the inner face of the stomodaeum and the proctodaeum. When this state is reached, the dorsal organ degenerates completely. Later a median dorsal organ, with lot of nuclear activity makes its appearance and at the same time the chitinous membrane becomes completely separate from the ectoderm in this area also. As a consequence, the embryo is now enclosed in three coverings, including the original egg membrane. The formation of this third membrane represents a precocious embryonic moult taking place as early as the nauplius stage.

Such processes of gastrulation and germ layer formation as is seen in *Panulirus* also take place in other decapods in a substantially similar way, although they may show more or less variation.

In contrast to the gastrulae of the Decapoda, which form a larger or smaller but distinctly invaginated blastopore, those of the Leptostraca and Peracarida exhibit no invagination. Gastrulation in these groups is effected by inward cell migration from the blastoporic area, which is at the same time covered over as the anterior lip of the blastopore grows backward. The mesoderm and endoderm cells, as well as the primordia of the gonads are occasionally distinguishable from the other elements before they move inward.

Presumptive Germ Layer Areas on Embryonic Surface

The endoderm cells are found to arise from D^{II} blastomere of the four-cell stage. This blastomere is one of the two which make contact at the vegetal pole, and is distinguished from the others by abundance of yolk, and peculiar type of granules. The mesoderm, stomodaeum and proctodaeum are conservative in their formation and fates throughout the Crustacea. An early segregation of primordial germ cells as a definite presumptive area takes place in the branchipods, copepods, ostracods and peracaridan Malacostraca, but not in the cirripedes or other Malcostraca. As in other major groups in which the time of first visible differentiation of the primordial germ cell varies, it is not possible to say whether early or late segregation of germ cells is the primitive.

Taking the Cirripedia as a starting point for consideration, the presumptive areas are described here (Fig. 10.3.18).

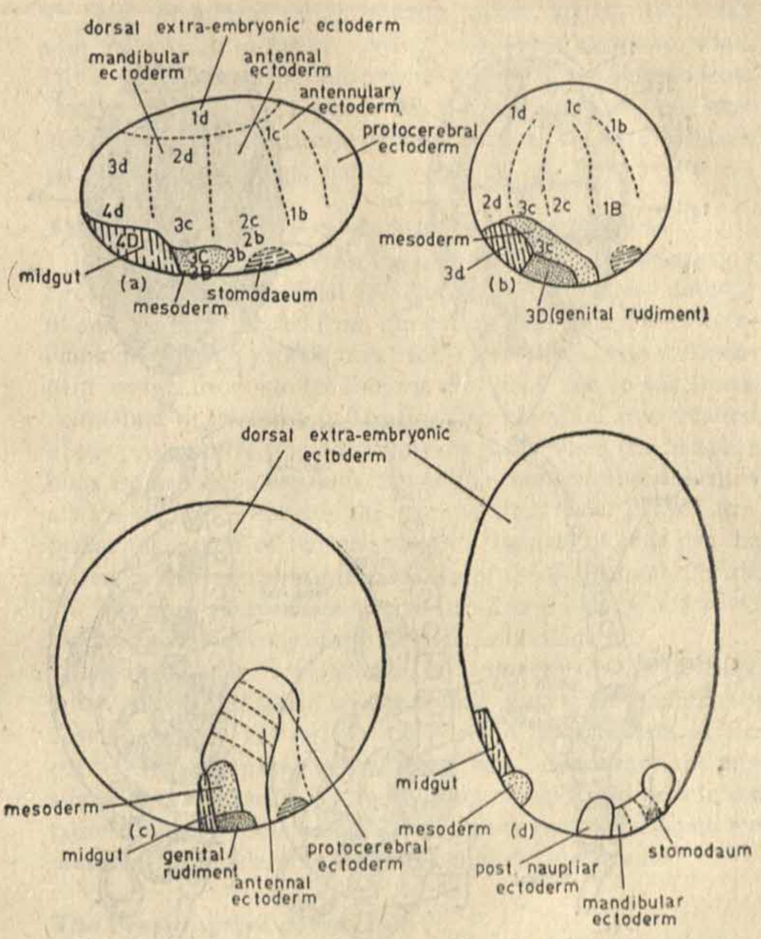


Fig. 10.3.18. Fate maps of crustacean blastula or blastoderm.
A—Cirripedea.
B—Cladocera and Copepoda.
C—Pericardian *Hemimysis.*
D—Eucardian *Caridina.*

The Presumptive Midgut

In the normal cirriped blastula, the presumptive midgut comprises the single, large, yolky cell, 4D. This cell fills the interior of the blastula, but is exposed at the surface postero-ventrally (Fig. 10.3.19). The immediate next step in the division of the

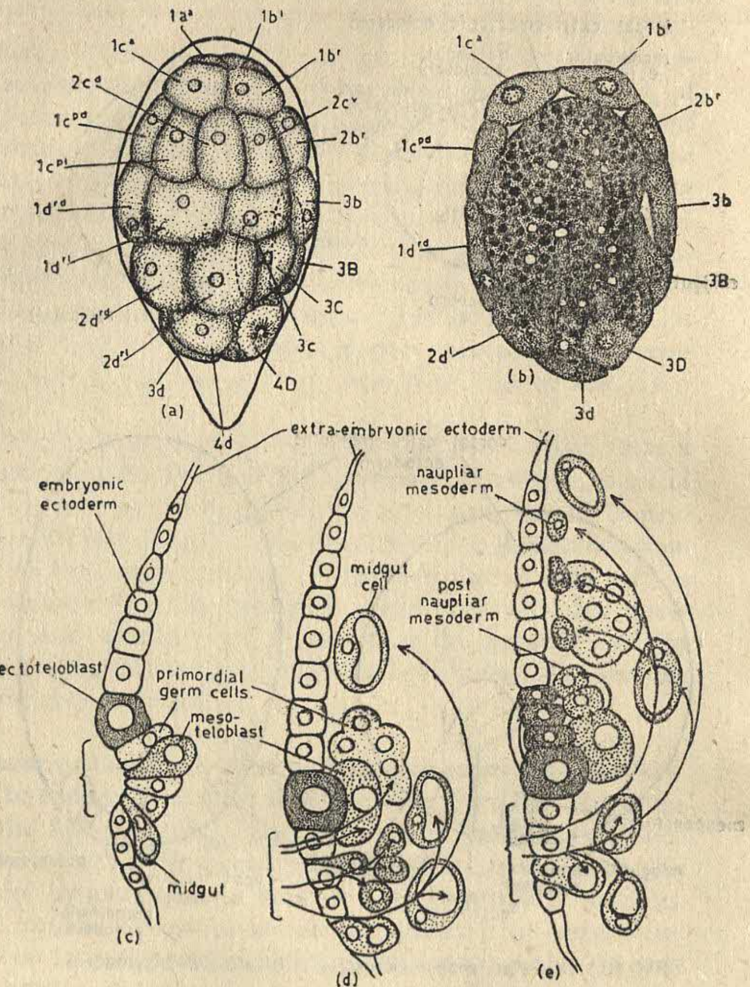


Fig. 10.3.19. A—31-33 cell stage of *Tetraclita* showing onset of mesodermal immigration.

B—Sagittal section, through the stage shown in 'A'.

C-E—Diagrammatic sagittal sections showing gastrulation in *Hemimysis*.

C—Onset of gastrulation.

D—Gastrulation in progress.

E—Gastrulation complete except for continued immigration by midgut cells.

presumptive midgut cell is an equal subdivision into two yolky cells, followed by a further division which segregates two small, yolk-free cells at the postero-ventral surface of the blastula from two large, yolky cells which fill the interior. The two large yolk cells are the anterior midgut rudiment. The two small cells are the posterior midgut rudiment.

The Presumptive Stomodaeum and Proctodaeum

In all crustacean embryos, the presumptive stomodaeum is a circular area of superficial cells in the ventral midline, anterior to and widely separated from the presumptive midgut. In later development, the presumptive stomodaeum makes an independent invagination into the interior and gives rise to the lining epithelium of the foregut. The first thickening and invagination of the presumptive stomodaeum takes place when the naupliar buds are just beginning to form and the stomodaeal rudiment is always seen to lie between the antennal limb buds. In the Cirripedia, the origin of the presumptive stomodaeal cells can be traced to the ventral, superficial cells of the B quadrant, 2b and 3b. The presumptive stomodaeum in all crustaceans is formed by the equivalent cells of the blastula or blastoderm.

The presumptive proctodaeum in Crustacea always develops late, after the naupliar segments have undergone preliminary development. It lies midventrally behind the ectoderm of the pre-telsonic, segmental growth zone. In all crustaceans, the presumptive proctodaeum is a small group of superficial cells in the postero-ventral midline of the presumptive ectoderm behind the presumptive ectoderm of the post-naupliar growth zone.

The Presumptive Mesoderm

The presumptive mesoderm of crustacean embryos varies little with the size and yolk content of the egg. The presumptive mesoderm in Cirripedia lies in the ventral midline as an area of superficial cells in front of the presumptive midgut. The three cells which comprise this area are the 3A, 3B and 3C. The 4d cell has no role in presumptive mesoderm formation. The cells migrate into the interior, undergo further divisions and move in a posterior direction to lie behind the midgut rudiment at the posterior end of the embryo. From here, they proliferate a pair of lateral bands of mesoderm cells forwards on either side of the midgut. The cells of the bands aggregate as three pairs of naupliar somites, leaving a residual posterior mass which gives rise to all of the post-naupliar mesoderm.

In larger eggs such as those of most Malacostraca, in which the presumptive mesoderm is a blastodermal area of cells in front of the presumptive midgut at the surface of the yolk mass, this pattern of further development is a little changed. The cells of the presumptive mesoderm move beneath the surface and are overgrown from in front by the presumptive ectoderm. The presumptive mesoderm then proliferates the paired mesoderm bands which break up into the three pairs of naupliar somites, and persists as a residual post-naupliar mass from which all of the post-naupliar mesoderm arises.

The Presumptive Ectoderm

The presumptive areas described above (midgut, stomodaeum, proctodaeum and mesoderm) lie along the ventral midline of the crustacean blastula or blastoderm and occupy a relatively small proportion of the surface. The remainder of the surface layer of cells is presumptive ectoderm, divisible between presumptive embryonic ectoderm adjacent to the ventral areas and presumptive extra-embryonic ectoderm more dorsally. The basic cells making up the presumptive ectoderm are the cells of the first three quartets 1a-1d, 2a-2d and 3a-3d, together with 4d. The only other components deriving from any of these cells are the presumptive stomodaeum of 2b and 3b origin and the presumptive proctodaeum, probably of 4d origin. The presumptive ectoderm develops as the ectoderm of the acron, labrum, naupliar segments and post-naupliar region, all of which can be presumptively zoned with the blastula as presumptive embryonic ectoderm.

Gastrulation

The gastrulation in the Crustacea is a relatively minor aspect of development. In the Cirripidia it begins when the fifth cleavage division has been completed. The first event of gastrulation (Fig. 10.3.19) is an inward and forward migration of the three presumptive mesoderm cells 3A, 3B and 3C beneath the ventral ectoderm cells in front of them. While this is happening, the yolk cell 4D divides equally into two cells by an approximately longitudinal division and then cuts off the two small, posterior midgut cells at the postero-ventral surface; the two presumptive posterior midgut cells, which lie behind the presumptive mesoderm now perform a gastrulation movement similar to the mesoderm, slipping inwards and forwards to join the mesoderm cells in the interior. By this time the three mesoderm cells have begun to divide. The presumptive embryonic ectoderm bordering the presumptive mesoderm and posterior midgut spreads to cover the small area of ventral surface vacated by the immigrating mesoderm and midgut cells. The major events of gastrulation are then complete and the external surface of the embryo retains no surface openings. The gastrulation movement of the presumptive stomodaeum is a later, independent invagination (Fig. 10.3.20) and that of proctodaeum, independent and still later. The small group of posterior midgut cells, which are distinctly round and pale staining, settle in a position immediately posterior to the yolky, anterior midgut cells. The mesoderm fills the posterior end of the gastrula below and behind them. The presumptive ectoderm, undergoes no gastrulation movements. Gastrulation movements remain much more distinct throughout the Crustacea in respect of the presumptive mesoderm. The cells of the presumptive mesodermal area migrate beneath the surface of the blastoderm and are overgrown by ectoderm from infront. The presumptive stomodaeum exhibits a uniform gastrulation movement throughout the Crustacea. The presumptive stomodaeum invaginates by an independent thickening and infolding (Figs. 10.3.20, 10.3.21 and 10.3.22) after the gastrulation movements of the mesoderm and midgut have been completed.

As the mesodermal and midgut rudiments complete their entry into the interior and the presumptive ectoderm spreads to replace them at the surface, the embryo begins to show the first signs of those changes in external form which will define the naupliar rudiments at the surface of the body and lead to the further elaboration of external structure. The external development of crustaceans after gastrulation is a highly complex and varied process, since it includes the details of functional larval elaboration and metamorphosis in species with indirect deve-

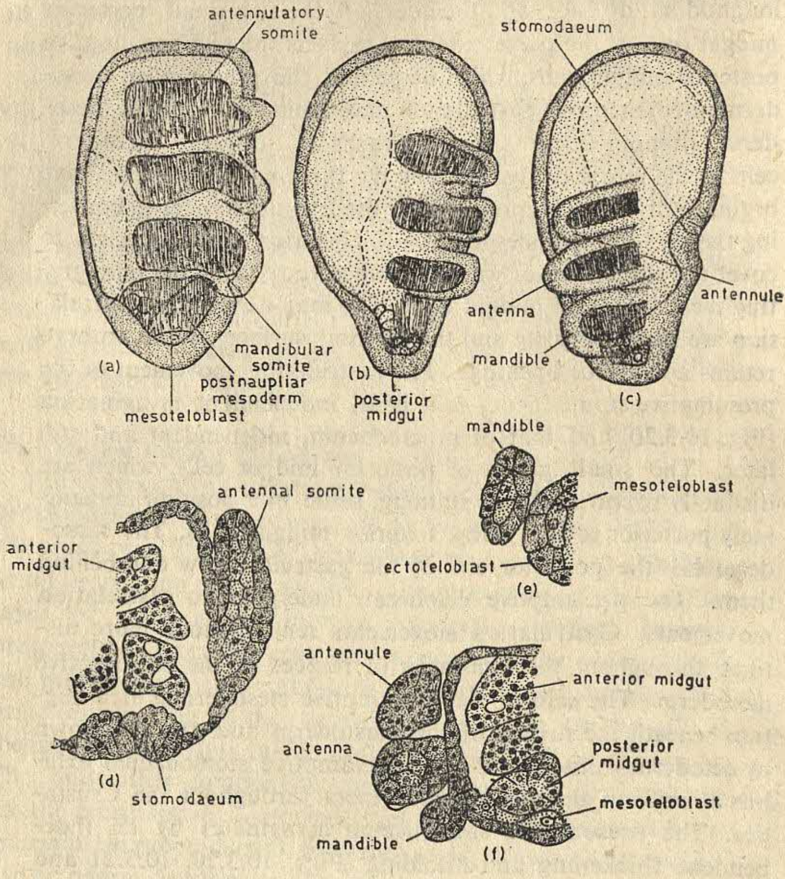


Fig. 10.3.20. A-F—Development of mesoderm in the embryo of *Tetraclita.*

A-C—Distribution of the naupliar somites and post-naupliar mesoderm during early development of the naupliar limb-buds.

D—T.S. through the embryo as shown in 'E' at the level of the stomodaeum.

E—T.S. at the level of caudal papilla.

F—T.S. after gastrulation.

lopment and the retention of embryonic anamorphosis even in those species with direct development.

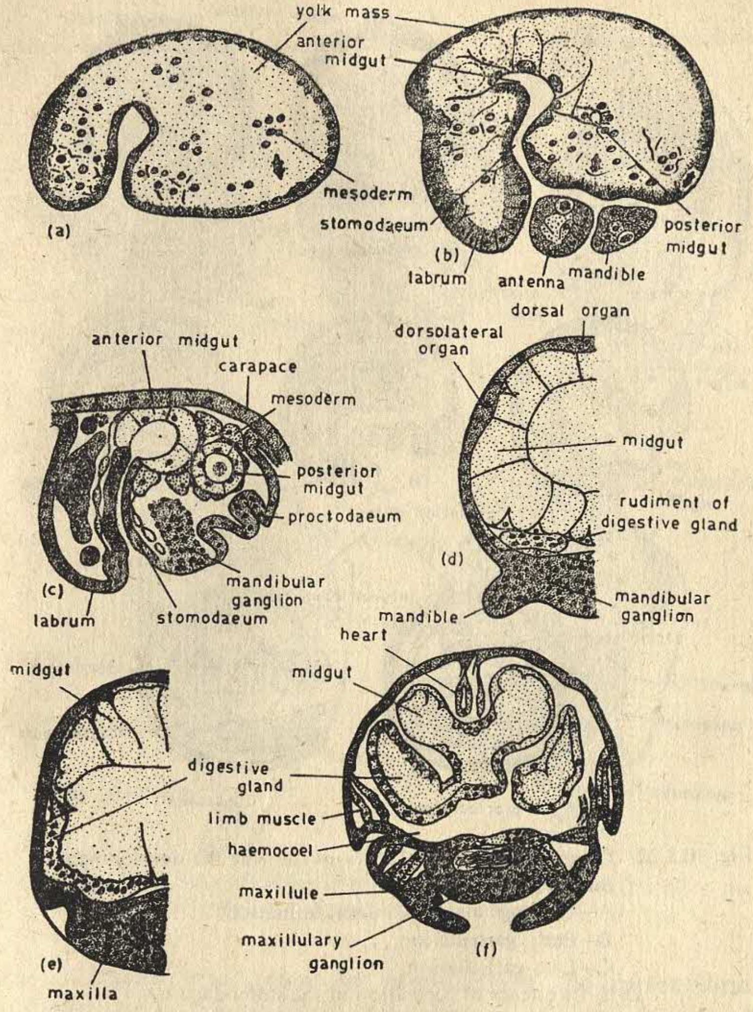


Fig. 10.3.21. A-C—Development of midgut in *Cyprideis*.
D-F—Development of midgut in *Hemimysis*.

Development of External Form

In those crustaceans which hatch as a nauplius larva, the external development of the embryo before hatching is in general terms, a relatively simple process. The first notable change is the development of the three pairs of naupliar limb buds, ventrolaterally along the sides of the body. As in Figs. 10.3.23

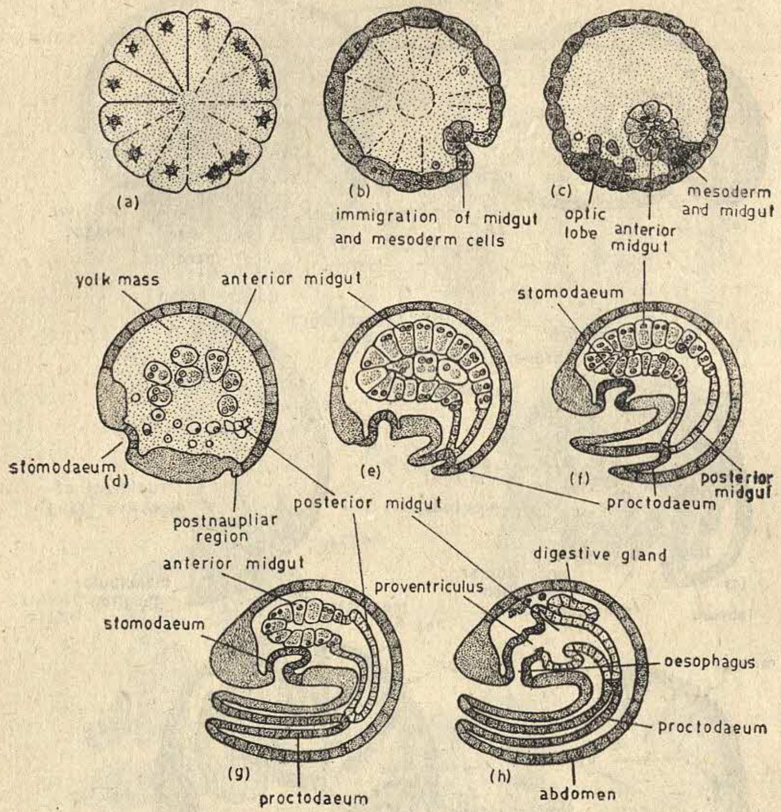


Fig. 10.3.22. Diagrammatic sagittal sections showing the development of the gut of *Galathea*.
A—Cleavage and blastoderm formation.
B—Early gastrulation.
C—Late gastrulation.
D-H—Sequence of formation of the stomodaeum.

and 10.3.24 the naupliar limb buds of cirripede and ostracod embryos occupy the major part of the body length indicating that most of the embryo is made up of the rudiments of the three naupliar segments, antennulary, antennary and mandibular. A relatively short, pre-antennulary region completes the anterior end and an even smaller post-naupliar region completes the posterior end. Other than the limb buds, the embryo does not manifest any external signs of segmental subdivision.

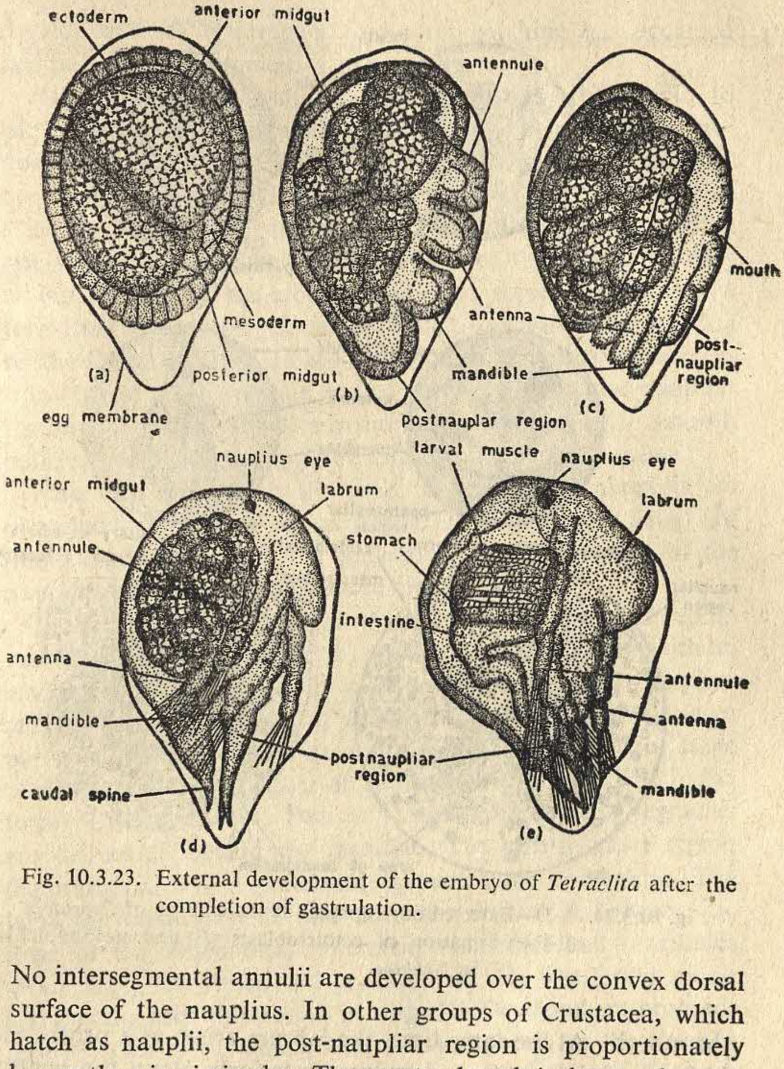


Fig. 10.3.23. External development of the embryo of *Tetraclita* after the completion of gastrulation.

No intersegmental annulii are developed over the convex dorsal surface of the nauplius. In other groups of Crustacea, which hatch as nauplii, the post-naupliar region is proportionately longer than in cirripedes. The greater length is due to the precocious development of the rudiments of several of the anterior post-naupliar segments in the embryo. As development of the embryo continues, the naupliar limb buds elongate and protrude, usually in a posteroventral direction (Fig. 10.3.23). The base of the antennal and mandibular limb buds also move forwards and downwards, so that they become crowded around

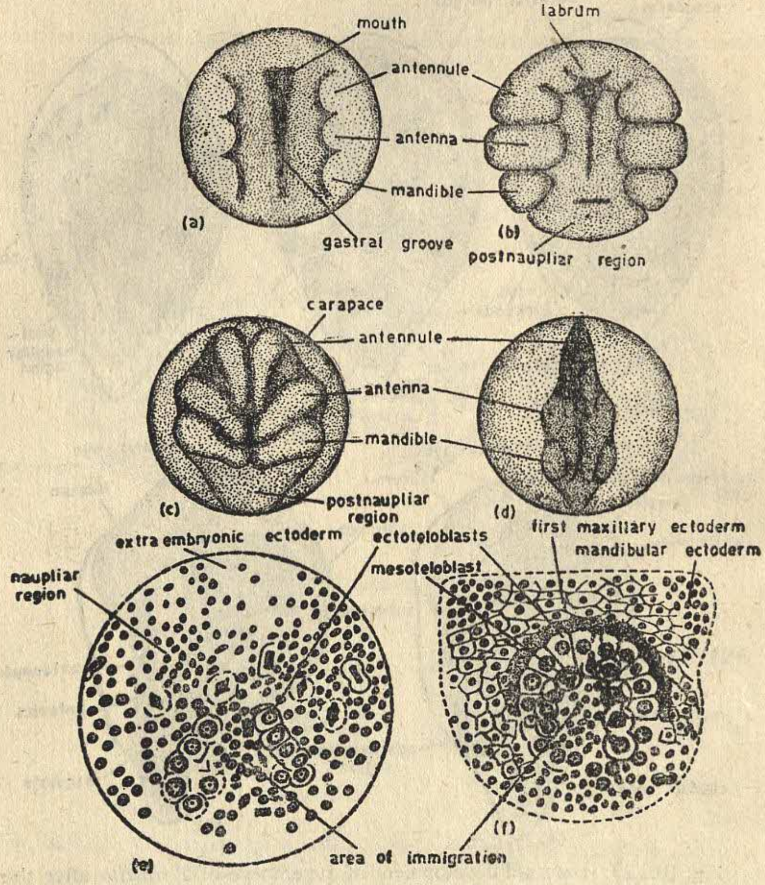


Fig. 10.3.24. A-D—External development of the embryo of *Cyprideis*. E-F—Formation of ectoteloblasts (E) and mesoteloblast (F) in *Nebalia*.

the mouth. At the same time, the labrum arises as a protrusion in front of the mouth and bulges posteriorly along the ventral midline to overhang the mouth. The bases of the antennules lie on either side of the base of the labrum, the bases of the antennae lie on either side of the mouth and the bases of mandible lie on either side just behind the mouth. Associated with the ventral concentration of the limb buds and labrum in the embryo nauplius, the dorsal surface of the embryo becomes more convex. In the majority of nauplii, there is no sign of the development of a carapace fold until hatching has occurred and naupliar development is proceeding.

As the limb buds continue their elongation, they begin to show the external annulation indicative of subdivision into podomere, and the antennae and mandibles become biramous. Secretion of the stage I nauplius cuticle begins at about this stage, accompanied by the onset of pigmentation of the median eye (nauplius eye) in the anterior midline of the pre-antennulary region, in front of the labrum. Cuticular secretion includes the formation of a unitary carapace shield over the convex setation on the naupliar limbs. In most nauplii, the carapace continues smoothly over the anterior end of the body. The post-naupliar region of the body remains ovoid and is covered by a smooth cuticle, sometimes bearing a terminal pair of caudal spines.

After hatching, when the limbs of the stage I nauplius are extended and begin to act as propulsive organs, the details of limb setation and of the general external structure of the nauplius can be seen much more clearly.

In the development of *Hutchinsoniella macracantha*, a cephalocaridean, hatching occurs as a nauplius 0.7 mm long, with an elaborate naupliar region and conspicuous post-naupliar region. The anterior part of the post-naupliar region carries a pair of maxillules, which have attained the prefunctional stage of development and a pair of rudiments of the maxillae in the form of minute lobes. Two or three limbless trunk segments are delineated behind the maxillae. The post-naupliar region then terminates in a pretelsonic growth zone and telson. The larva still contains yolk and does not feed at this stage. The setae of the antennules, antennae, mandibles and maxillules attain their full functional condition at the first larval moult and feeding probably begins at stage II. Development proceeds through a series of moults and metamorphosis to the juvenile adult form takes place during the fourteenth and fifteenth moults, when the larvae becomes 1.8 mm long. By this stage, the full complement of nineteen trunk segments has already been developed.

The antennules show little change other than increase in size as development proceeds. During the naupliar stages, they are elongate, setose, uniramous limbs, which function as locomotory organs and also act in stirring up food particle from the substratum. The same form and function then persist after metamorphosis has occurred. The antenna also function in locomotion and feeding in the nauplius, but in a more complex manner than the antennules. Each antenna is long and biramous, with long setae on the exopod and shorter setae on the endopod. The naupliar form and function of the antennae persist, with increasing size, through fourteen moults. At the fifteenth moult metamorphosis of the antennae occurs. The distal protopodal setae and masticatory spine, which form part of the naupliar ingestive apparatus, are eliminated, but the remainder of the antennal form and function continue into the adult.

The mandibles function in locomotion and feeding in the nauplius in the same manner as the antennae. Later in naupliar development, at the twelfth moult, the base of each mandible enlarges to form a gnathobase which projects beneath the labrum towards the mouth. The exopod of the mandible become vestigial and the endopod is reduced. After two further moults, only the gnathobase of the mandible remains as the mandible of the adult. The naupliar functions of the mandibles in the Cephalocarida are thus lost at metamorphosis and are replaced by masticatory function.

In the Cephalocarida, unlike other Crustacea, a serial functional development of the post-naupliar region accompanies the enlargement of the naupliar region during the moults which precedes metamorphosis.

The two regions of the body are linked functionally by the maxillules, which become functional with the onset of feeding after the first moult. At this stage, the maxillules are structurally similar to the trunk limbs which subsequently develop behind them. The exopod is flattened and has a natatory function, supplementing the locomotory action of the naupliar limbs. The endopod is cylindrical and has an ambulatory and food-stirring function. The protopod carries a number of median endites which assist in pushing food particles forwards beneath the labrum. This form and function persists through the first nine stages of naupliar development. Then at the ninth moult, the basal endite of each maxillule shows its first sign of enlargement as a gnathobase and the remainder of the limb is slightly reduced. This trend continues during several moults, until, by the time metamorphosis is complete, the gnathobase is fully elongated and the remaining endites have been lost. The expods of the maxillules, retain their natatory function after metamorphosis but the endopods assume a new orientation and function at this stage. They are no longer ambulatory but now serve to close off the sublabral space laterally. This change is functionally associated with the transition from naupliar feeding to feeding exclusively in the adult manner by transferring food forwards from the trunk limbs. The maxillae are structurally and functionally identical with the succeeding trunk limbs. The maxillae becomes functional after the third naupliar moult, when the naupliar limbs and maxillules are already active in locomotion and feeding. Their function is in the forward transfer of food particles on to the endites of the maxillules. This form and function persists through metamorphosis.

The trunk limbs develop slowly in a serial manner behind the maxillae. Eight pairs of trunk limbs are developed in *H. macracantha* leaving eleven limbless trunk segments at the posterior end of the body. The first pair become functional coincident with the maxillae at the third moult, but the eighth pair does not become functional until the twentieth moult. Each pair of trunk limbs resembles the maxillae in form and function, and contributes to locomotion, food collection and the forward transfer of food to the maxillules.

At the fifteenth moult, the stage at which the fifth pair of trunk limbs becomes functional, the naupliar ingestive apparatus is lost and the mandible and maxillules assume their adult form and function.

All of the other main groups of Crustacea which hatch as nauplius larvae are developmentally more specialized than the Cephalocarida. Firstly, their nauplius larvae are planktonic and except in penaeidae, planktotrophic. Secondly, the developmental sequences through which they pass after hatching are more specialized than that of the Cephalocarida and differ markedly in each group. In order to appreciate the nature of these specializations, it is necessary to discuss each group in turn.

The newly hatched nauplius of the Branchiopoda, *Artemia* has an elaborate naupliar region and a conspicuous post-naupliar region. The naupliar region (Fig. 10.3.25) is structurally and functionally specialized in relation to active swimming and

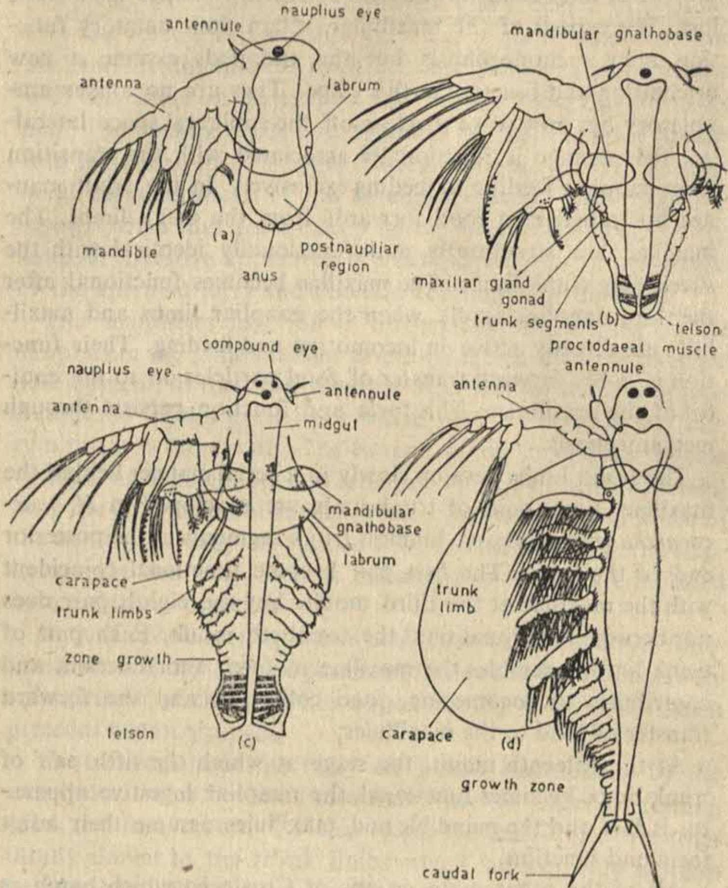


Fig. 10.3.25. A-D—The larval development and metamorphoses in *Limnadia*.
A—Stage 1 nauplius.
B—Stage 3 nauplius.
C—Stage 5 nauplius.
D—Newly metamorphosed juvenile stage 6.

planktotrophic feeding, while the post-naupliar region is externally simplified. The structural and functional elaboration of the naupliar region is completed during the first three larval stage and feeding begins during stage III. The newly hatched nauplius of *Artemia* is 450 μ and when feeding begins this increases to 725 μ. Naupliar development continues through a further five stages, during which the post-naupliar region increases in length and becomes further elaborated. Metamorphosis to the juvenile adult form then takes place at the ninth moult, the resulting juveniles being about 2 mm long.

The full structural differentiation of the naupliar region is attained by the time feeding begins. The uniramous antennules are small and project forwards as sensory processes. The antennae are long, biramous limbs with long, hinged setae on the exopod and endopod. The mandibles are short and uniramous but also carry terminal hinged setae. The antennules play no part in locomotion but the antennae with their terminal fans of setae, act as the major propulsive organs of the nauplius. Their action is oar-like within the setal fans spread during the forward stroke. An associated oar-like action of the mandible also assists propulsion through the water. Swimming in branchiopod nauplii is also accompanied by feeding on particulate matter in suspension in the water.

The sharp transition from naupliar feeding to adult feeding at metamorphosis in the Branchiopoda is in marked contrast to events in the Cephalocarida. The development of the branchiopod post-naupliar region is modified in such a way, that the first six pairs of trunk limbs become functional simultaneously, coincident with loss of the specialized naupliar ingestive apparatus. The antennae lose their food collecting function and the mandibles become reduced to gnathobases.

The specialization of development of the branchiopod postnaupliar region is evident even at hatching. Although not marked externally by intersegmental annuli or limb buds at this stage, the region already includes the rudiments of the first eight postnaupliar segments (the maxillulary, maxillary and first six trunk segments) together with a pretelsonic growth zone and terminal telson which carries the anus. The maxillulary and maxillary segments remain vestigial, but the first six trunk segments develop rapidly and more or less synchronously, exhibiting limb buds at stage IV. The six pairs of limb buds become functional, coincident with metamorphosis, but the development of the seventh and succeeding trunk segments and their limb buds is relatively delayed (Fig. 10.3.25).

In branchiopods, therefore, the development commences with the formation and hatching of a nauplius, as in the Cephalocarida, but is adapted to a more direct attainment of the first functional stage at which feeding can proceed by trunk limbs alone, that is, the stage with six pairs of functional trunk limbs. The adaption comprises specialization of the naupliar locomotory and feeding apparatus, a synchronous, precocious development of the first six trunk segments and sudden transition from one to the other at metamorphosis and fewer naupliar stages than in the Cephalocarida.

The groups Cirripedia and the Copepoda exhibit a parallel neotenic condition of six pairs of trunk limbs as the basic adult number. After hatching, the larva (Fig. 10.3.26) passes through six naupliar stages with intervening moults. The stage I nauplius, which usually have a length of 200-400 μ subsists on yolk and moults within two hours of hatching. Planktotrophy begins at stage II and persists, with retention of the naupliar swimming and feeding apparatus, until stage VI. The stage VI nauplius usually varies between 500 μ and 1200 μ in length in different species. At the sixth moult, the larva metamorphoses to a cypris (Fig. 10.3.27), in which the naupliar swimming and feeding apparatus are lost, feeding is temporarily suspended, and swimming is performed by six pairs of biramous trunk limbs. After a period of swimming, the cypris settles. Metamorphosis to the adult form is then attained through a further moult.

During the naupliar phase of development, the naupliar region makes up most of the body. The antennules are uniramous, but unlike those of the branchipod nauplius, are long, setose limbs and are used as swimming organs in conjunction with the antennae and mandibles. The biramous antennae, which carry long setae on the exopod and endopod are the main propulsive organs. The long biramous mandibles also act as propulsion organs, but are the main food collecting limbs of the nauplius. Particles in suspension in the water are gathered mainly by the long setae of the mandibular endopods and swept beneath the ventral surface of the nauplius. The cuticle of this surface bears areas of short, denticulate spines, which appear to scrape the particles from the mandibular setae during the next forward stroke of the mandibles. During the back-

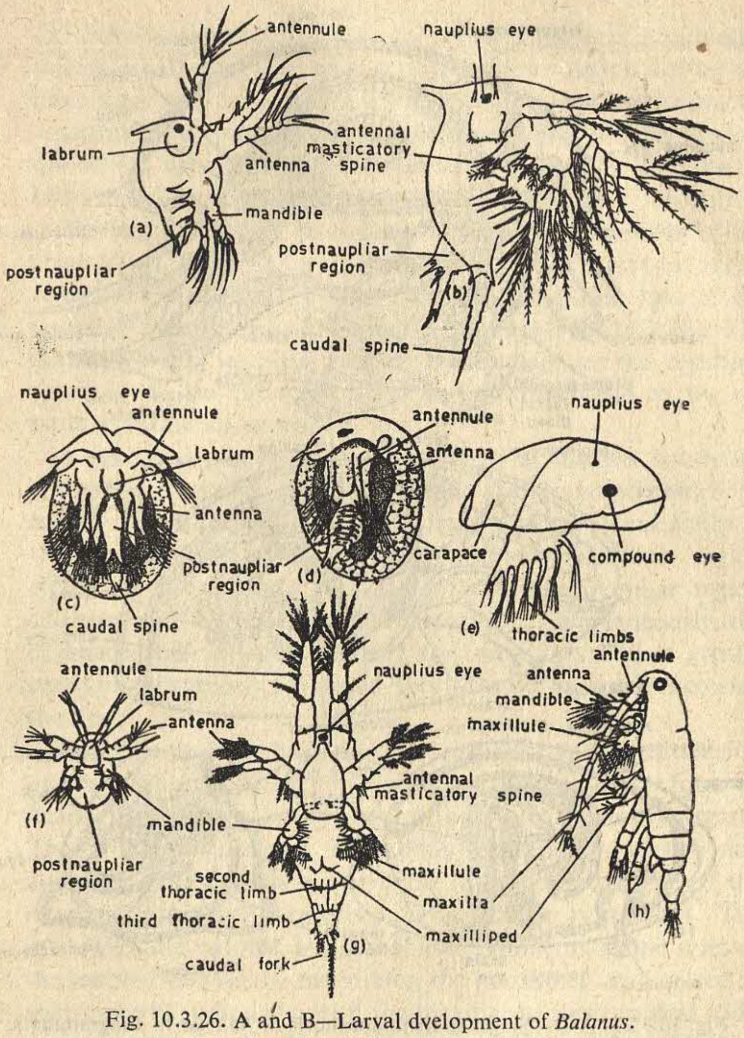


Fig. 10.3.26. A and B—Larval dvelopment of *Balanus*.

A—Stage 1 nauplius.
B—Stage 5 nauplius.
C-E—Larval development and metamorphosis in *Ibla*.
C—Stage 1 nauplius.
D—Stage 5 nauplius.
E—Cypris stage.
F-H—Larval development and metamorphoses in *Labidocera*.
F—Stage 1 nauplius.
G—Stage 6 nauplius.
H—Stage 6 copepodid.

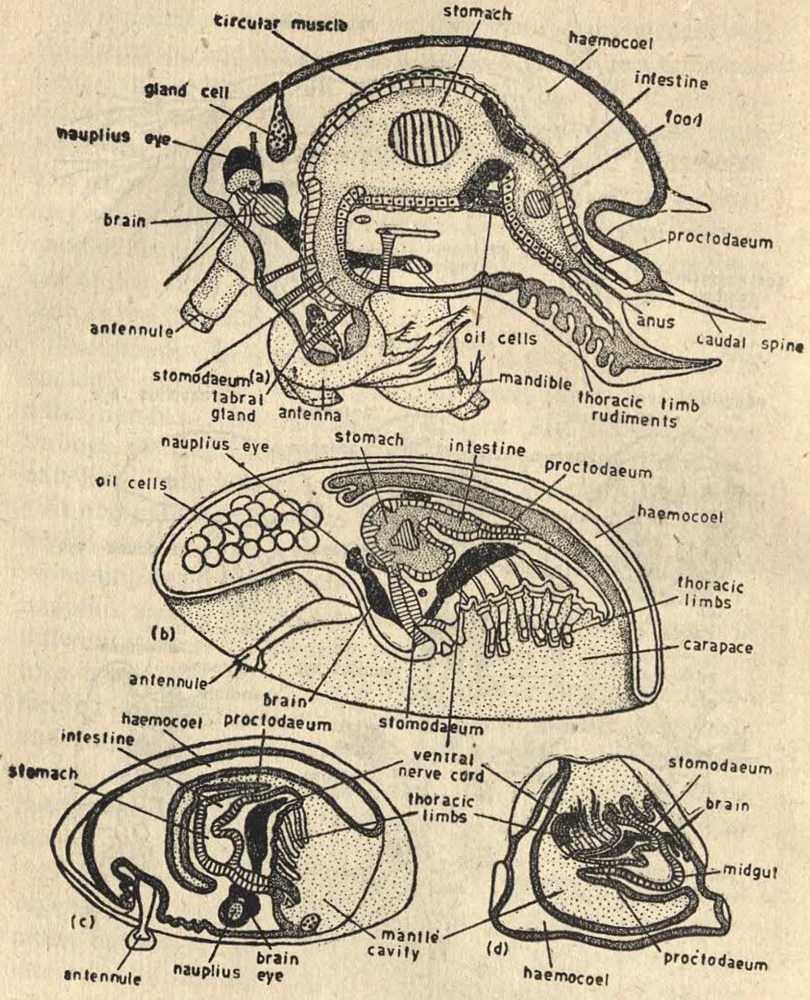


Fig. 10.3.27. A-D—Stages in metamorphosis of *Balanus* in diagrammatic sagittal section.
A—Fully developed stage 6 nauplius.
B—Cypris stage.
C & D—Completion of metamorphosis after setting.

stroke of the mandibles, these processes assist in the forward transfer of food particles towards the mouth.

The development of the post-naupliar region, just as the naupliar region, can be interpreted as functional modification of the cirripedes, and differs from the branchiopods in retaining a sequential development of the post-naupliar region during the nauplius stage. At hatching the cirripede post-naupliar region comprises little more than a pretelson growth zone. The Cirripedia have a specialized development of limb bands. The maxillules, maxillae and the first six pairs of trunk limbs develop more or less simultaneously as rudiments beneath the cuticle of the stage VI nauplius and become exposed simultaneously at metamorphosis at a single moult, coincident with the loss of the naupliar swimming and feeding apparatus (Fig. 10.3.27). The maxillules and maxillae remain rudimentary in the resulting cypris larva, but the trunk limbs become functional as the six pairs of biramous swimming legs.

A detailed study of the metamorphosis of *Balanus balanoides* shows that a complex series of external changes accompanies the exposure and onset of function of the six pairs of trunk limbs at the nauplius-cypris moult. While the post-naupliar region elongates and become newly functional, the naupliar region shortens and is functionally transformed. Simultaneously, the carapace fold extends laterally and anteriorly and is greatly extended posteriorly to form a deep bivalve carapace enclosing the body.

The antennules, which now lie within the anterior extension of the carapace fold, remain large, but take on a new form as flexed, forwardly pointing limbs with terminal adhesive organs. Their functions are now in walking, exploratory sensing and adhesion. The antennae, the main locomotory organs of the nauplius, are wholly resorbed. The labrum is reduced. The mandibles are reduced to gnathobasal rudiments at the sides of the mouth. Behind the mandibles, the maxillules are exposed as ventrolateral rudiments, with the maxillae as short ventral rudiments between them. None of the mouthparts is functional at this stage. The mouth is closed and feeding is suspended temporarily. Behind the maxillae, the six pairs of trunk limbs are well developed as locomotory organs.

When settlement by antennular attachment has been achieved, the cypris moults again and undergoes a change in form (Fig. 10.3.27). The anterior extension of the carapace fold is eliminated. The body is rotated through 180°, bringing the posterior ventral opening of the mantle cavity to the apex of the now upper surface and the trunk limbs to a position in which they project upwards rather than downwards. With this change, the synchronous action of the six pairs of trunk limbs becomes a feeding action. The mouth is reopened, the mouth parts become functional and feeding begins in the adult manner.

The metamorphosis is unique in Cirripedia. Much of the specialized development preliminary to metamorphosis takes place in the last nauplius stage.

At the time of hatching, the copepod larva (Fig. 10.3.26) has an elongate ovoid body, lacking the carapace spines of the cirripede nauplius. Development proceeds through six naupliar stages, with feeding commencing at stage III. During these stages, the naupliar region enlarges and the post-naupliar region increases in length and displays the beginnings of external segmentation and limb buds. At the sixth moult, metamorphosis occurs to a first copepod stage, a juvenile form in which the first five post-naupliar segments become functional. Further development proceeds through a total five copepod stages before the moult to the adult takes place. The locomotory and feeding action of the copepod nauplius are basically like those of the cirripede nauplius. The uniramous antennules are long, setose limbs, sometimes held in a forward, pointing position and sometimes moving in unison with the antennae and mandibles. The biramous antennae, which have fans of setae on the exopod and endopod and a masticatory spine and other short setae on the median face of the protopod, are the main propulsive organs. The mandibles assist the antennae in propulsion, but also filter food particles from the water and sweep them beneath the body. The mandibles also develop functional gnathobases at the third naupliar moult, protruding forwards beneath the labrum as part of the naupliar ingestive apparatus. After the sixth moult, the antennules become further elongated and are held motionless or used for sudden jumping movements. The antennae become relatively reduced, lose their long swimming setae and ingestive setae and no longer act in ingestion. The mandibular gnathobases become enlarged, but the same are reduced and are no longer significant in locomotion. Both the locomotory and feeding function are shifted to the post-naupliar region.

The elongation of the post-naupliar region in the nauplius is accompanied by external demarcation of the maxillulary and maxillary segments at stage V and of the first two thoracic segments at stage VI. At metamorphosis to the first copepod, the region elongates further and the third thoracic segment is delineated. During the succeeding four copepod stages, the last four thoracic and five abdominal segments are bilineated. In general, segment determination takes place in a serial order.

The formation and onset of function of the post-naupliar limbs follows a more complex sequence. The maxillulary rudiments are first exposed in the stage IV nauplius, and the maxillary rudiments in the stage VI nauplius, but neither becomes functional until metamorphosis takes place. Both pairs then assume approximately their adult form and functions and become associated with the mandibles as components of the post-naupliar feeding apparatus. The second and third thoracic segments are exposed as functional swimming limbs between stage VI nauplius to stage I copepod. The fourth, fifth and sixth pairs of thoracic limbs become functional as swimming limbs in the third, fourth and fifth copepodid stages of development respectively.

In copepods, therefore, the pattern of specialization in the development of the post-naupliar region is different from that of cirripedes, in spite of the similarities in the naupliar region. Associated with the planktotrophic specialization of the naupliar region, the post-naupliar region develops rapidly and metamorphosis occurs at an early moult. Before metamorphosis, the post-naupliar region is not functional in locomotion and feeding. At metamorphosis, it takes over these functions. The takeover involves: (1) A simultaneous development of the maxillules, maxillae and first thoracic limbs (maxillipeds) as a post-naupliar feeding apparatus, associated with metamorphosed mandibles, and (2) A coincident, specialized development of the second and third pairs of thoracic limbs as specialized swimming limbs. The functional specialization of the mouth parts and the first three pairs of trunk limbs in this way obviates the need for the establishment of six pairs of functional trunk limbs as a prerequisite to moving and feeding in the adult manner. Associatedly, the serial development of the last three pairs of trunk limbs is delayed until after metamorphosis.

In the Malacostraca, there is no guiding principle of metamorphosis associated with the functional development of six pairs of trunk limbs. The basic sequence of larval development of the Malacostraca, as exemplified by the development of the Penaeidea, involves a complex series of larval stages (Figs. 10.3.28 and 10.3.29) and includes the development of eight throracic and six abdominal pairs of limbs.

Penaeid nauplii at hatching are larger than those of copepods and cirripedes and have a relatively long post-naupliar region. The nauplius stages of development which vary in number are sustained by yolk carried over from the egg. In association with this, they lack the antennal masticatory spines, antennal and mandibular protopodal setae and mandibular gnathobases of the naupliar ingestive apparatus but retain naupliar natatory limbs similar to those of copepods and cirripeds. During the naupliar stage, the post-naupliar region increases in length, due to the proliferation of the rudiments of at least ten post-naupliar segments. The nauplius of *Penaeus durarum* is about 600 μ long at stage V. The rudiments of the first four pairs of post naupliar limbs (maxillules, maxillae and first two trunk segments) are also exposed at the surface in the stage IV nauplius and become enlarged at stage V but do not become functional until the next moult.

With the next moult (Vth) the nauplius undergoes the first major step in the development of the post-naupliar region and becomes a protozoea. There is a considerable increase in length (about 1 mm) mostly in the post-naupliar region. The antennules and antennae retain their naupliar form and function, but the mandibles are reduced to functional gnathobases. The carapace fold is also exposed for the first time at this stage of development and extends back over the dorsal surface of the body as far as the second trunk segment.

In the post-naupliar region of the stage I protozoea, the eight thoracic segments are now delineated externally, though only the first two carry limbs. Behind the eighth thoracic segment is the abdominal growth zone in front of the forked telson. The maxillules and maxillae become elongate and setose and commence their function as ingestive limbs in association with mandibles. The first two pairs of trunk limbs develop as setose biramous limbs, the first and second maxillipeds, whose exopods

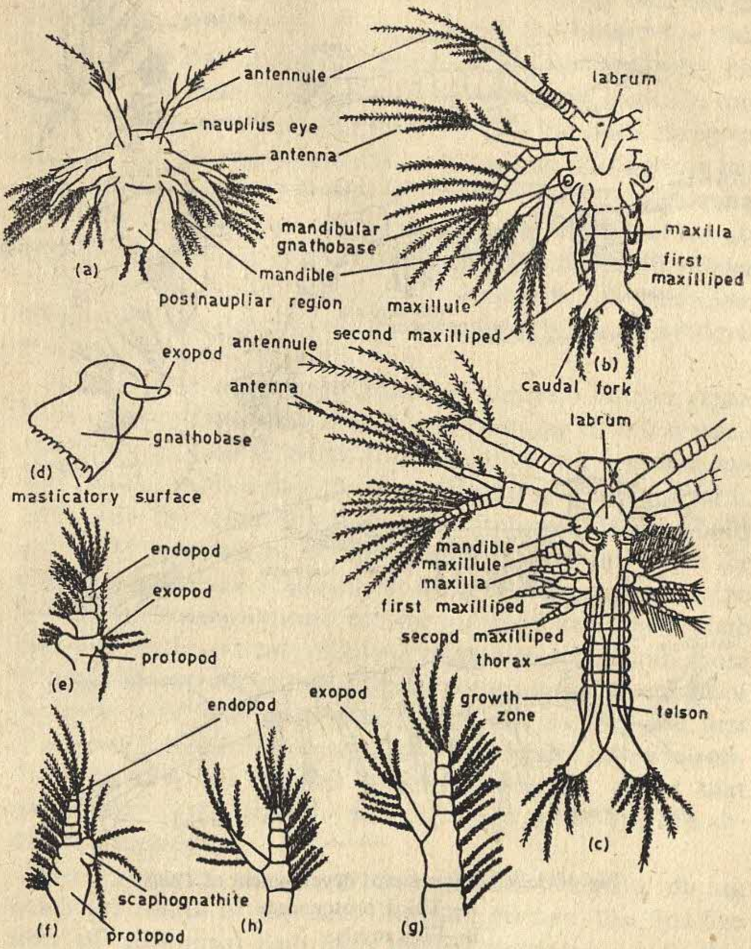


Fig. 10.3.28. A-C—Early development of *Penaeus*.
A—Stage 2 nauplius.
B—Stage 5 nauplius.
C—First protozoea.
D-H—Limbs of first protozoaea.
D—Mandible.
E—First maxilla.
F—Second maxilla.
G—First maxilliped.
H—Second maxilliped.

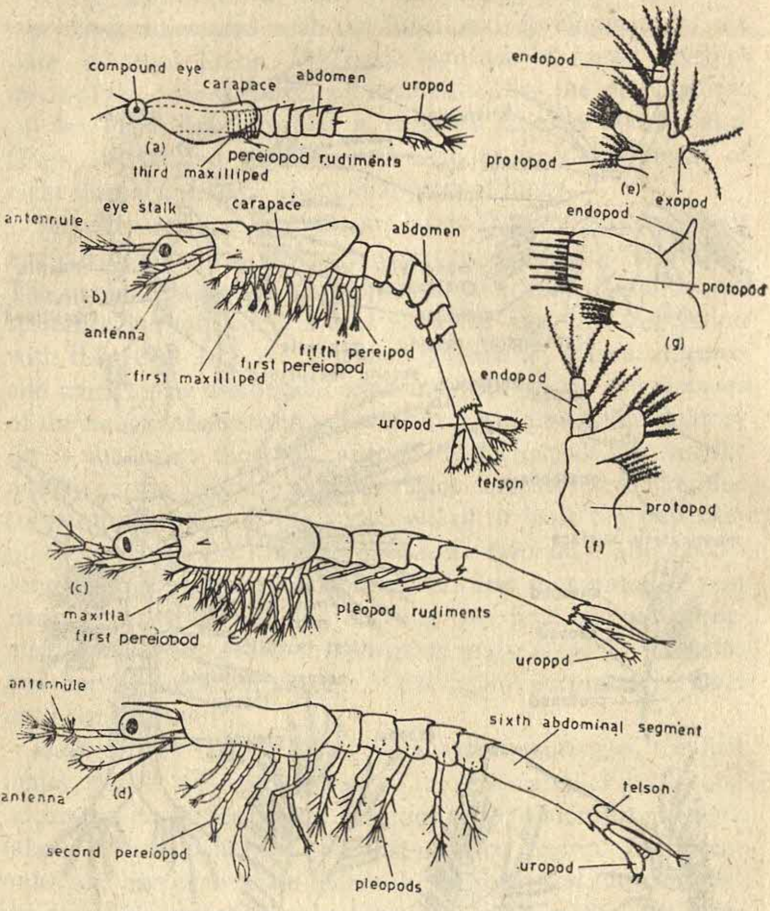


Fig. 10.3.29. Later larval development of *Penaeus*.
A—Third protozoaea.
B—First mysis.
C—Third mysis.
D—First post-larva.
E—First maxilla of third protozoaea.
F—First maxilla of third mysis.
G—First maxilla of first post-larva.

augment the swimming action of the antennae and whose endopods play a part in food capture.

Protozoeal form and function now persists through several moults during which the body length increases further. During this phase of development, the stalked compound eyes are exposed on the anterior part of the head and the carapace extends back to cover the posterior part of the thorax. The rudiments of the last six pairs of thoracic limbs are developed, but the thoracic segments on which they lie remain short. In contrast, the abdomen increases greatly in length, with demarcation of the six abdominal segments in front of the telson and show a precocious development of the sixth pair of abdominal limbs as functional uropods. The onset of function of the abdomen and tail fan as a locomotory organ for rapid sudden movement through the water is associated with this precocious development, but the first to fifth abdominal limbs are not developed during the protozoaea phase.

At its next moult, the protozoaea undergoes a further structural and functional transformation, emerging as a first mysis stage. The increase in length at this moult is not excessive but development takes a major step towards the juvenile adult form. Anteriorly, the antennules become relatively reduced and wholly sensory in function, as do the antennae. So at this moult the naupliar propulsive apparatus is lost. At the same time the last six thoracic segments become enlarged and their limb buds become functional, with long exopodites which join those of the first and second maxillipedes as the swimming organs of the mysis larva. The mandibles, maxillules, maxillae and first two pairs of maxillipedes retain their feeding and ingestive role. The long abdomen and tail fan are unchanged, except that rudimentary limb buds are now developed on the first five abdominal segments.

The mysis form is retained through several moults during which the length of the larva increases further. The first five pairs of abdominal limb buds become elongated during this phase, but do not become functional. The swimming and feeding processes of the larva remain unchanged until, at a single moult the larva undergoes a final transformation into a first post-larva or juvenile stage. At this moult, the mouth parts and maxillipedes assume their basic adult form, the fourth to eighth thoracic limbs become pereiopods with enlarged endopods and reduced exopods and the first five pairs of abdominal limbs become functional pleopods. The swimming function thus shifts at a single moult from the thorax to the abdomen.

Basic malacostracan development as exemplified by the penaeidae therefore exhibits the elimination of naupliar feeding but retains a strict antero-posterior sequence of post-naupliar development, combined, with a progressive shift of natatory function to successively more posterior groups of limbs as development proceeds. In spite of the similarities in naupliar locomotion, the pattern of later larval specialization of the Malacostraca is fundamentally different from those of cirripedes and copepods. The functional specialization of the Malacostraca before metamorphosis are:

1) Precocious development of the first four naupliar limb-pairs as a specialized feeding apparatus in association with mandibular gnathobase. This apparatus becomes functional at the moult from nauplius to protozoaea.

2) The associated retardation of development of the next six trunk segments and their limbs.

3) The precocious development of the last six trunk segments.

4) A retention and further elaboration of the feeding apparatus already established including the first two trunk limbs.

5) A sudden onset of function, emphasizing natatory exopods, of the next six pairs of trunk limbs, but no direct involvement of these limbs in feeding.

The later transition from thoracic to abdominal natation at the moult from mysis to post-larva is clearly a secondary specialization.

In the penaeid decapod, *Penaeus* we have seen that it hatches as a nauplius and passes through several nauplius, protozoaea and mysis stage before becoming a juvenile at the eleventh moult. In the brachyuran crab *Macropodia*, the nauplius and protozoaea stage are passed during embryonic developments. The stages are clearly recognizable in the embryo, the nauplius stage by its precocious development of the three naupliar segments and their limb buds, the protozoaea stage by its precocious development of the maxillules, maxillae, first and second maxillipeds and abdominal segments. But functional differentiation of the embryo proceeds directly to a hatching stage equivalent to the first mysis stage of *Penaeus* (compare Figs. 10.3.29 and 10.3.30). The continued suppression of the posterior thoracic region of *Macropodia* at the hatching stage is a specialized brachyuran feature. The development of *Macropodia* prior to first mysis equivalent has thus been embryonized and the functional

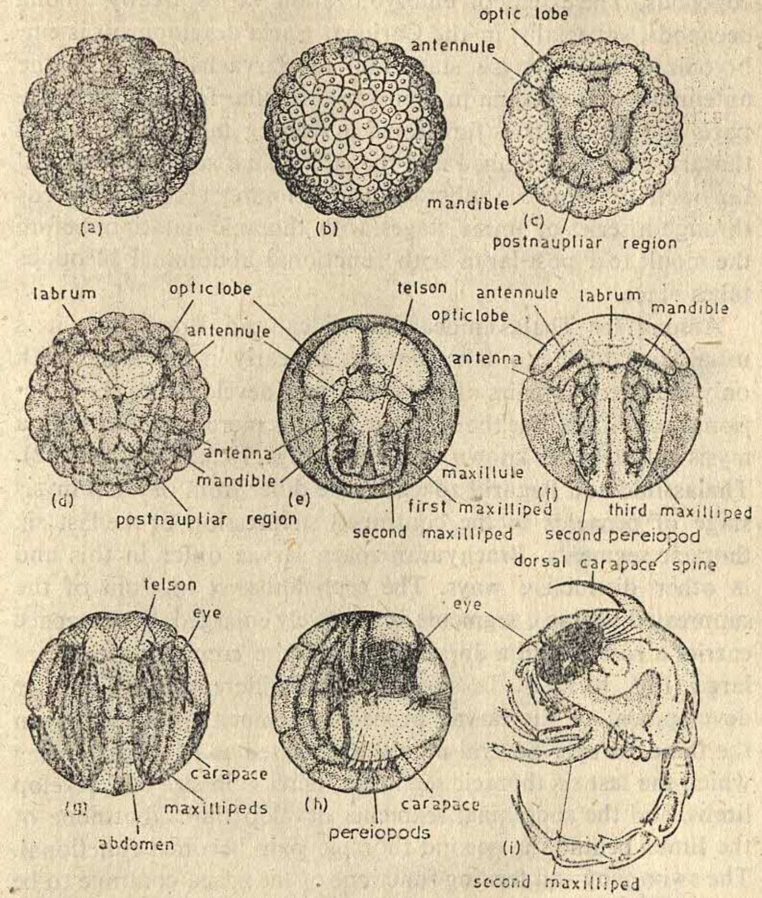


Fig. 10.3.30. External development of the embryo of *Macropodia*.
A—Cleavage.
B—Blastoderm.
C—Nauplius rudiment.
D—Formation of caudal papilla.
E & F—Formation of post-naupliar segments and limbs.
G & H—Completion of development.
I—Newly hatched first zoaea.

differentiation of ancestral larval components prior to this stage has been lost.

The embryonization of development in the Malacostraca is much more dive and far reaching than in cirripedes and copepods. The extent of embryonization varies greatly among decapods, especially in the Caridea. Carid development is embryonized to the mysis stage and the larva hatches with the antennules and antenna in their post-naupliar form, the mouthparts and maxillipedes functional in feeding and swimming, all thoracic segments defined and the abdominal segments and tail fan well developed. Subsequent development then proceeds through a series of mysis stages with thoracic natation, before the moult to a post-larva with functional abdominal pleopods takes place.

Among the Thalassinidea, Paguridea and Brachyura, it is usual for hatching to take place at an early mysis stage, with only the first two pairs of thoracic limbs developed to the functional condition, but the hatched larva is more modified than a mysis and is better known as a zoaea (Figs. 10.3.31 and 10.3.32). Thalassinid and Pagurid zoaea larvae differ from the first mysis stage of penaeids in the continued suppression of the last six thoracic segments. Brachyuran zoaea larvae differ in this and in other distinctive ways. The cephalothorax in front of the suppressed thoracic segments is relatively enlarged, the carapace carries a rostral and a dorsal spine and the compound eyes are large (Fig. 10.3.32). In spite of these differences, the further development of the larvae proceeds in much the same way in the three groups. Several moults are passed as a zoaea, during which the last six thoracic segments increase in size and develop limbs, and the abdominal segments develop limbs, but none of the limbs behind the second thoracic pair becomes functional. The swimming and feeding functions of the zoaea continue to be carried out by the mouthparts and the first and second maxillipeds, with assistance from the precociously developed abdomen and tail fan. Then at a single moult, the zoaeal swimming function is lost, the thoracic limbs become functional in their adult form and the abdominal limbs become functional as pleopods. This larval stage which is a post-larva in the Thalassinidea but is called a glaucothoe in the Paguridea and megalopa in the Brachyura, remains free swimming, but immediately precedes the first juvenile stage attained at the next moult.

Only in the Palinura, among the decapods there is a more extreme larval specialization evolved (Fig. 10.3.31). Hatching occurs at a relatively advanced stage, in which the antennules

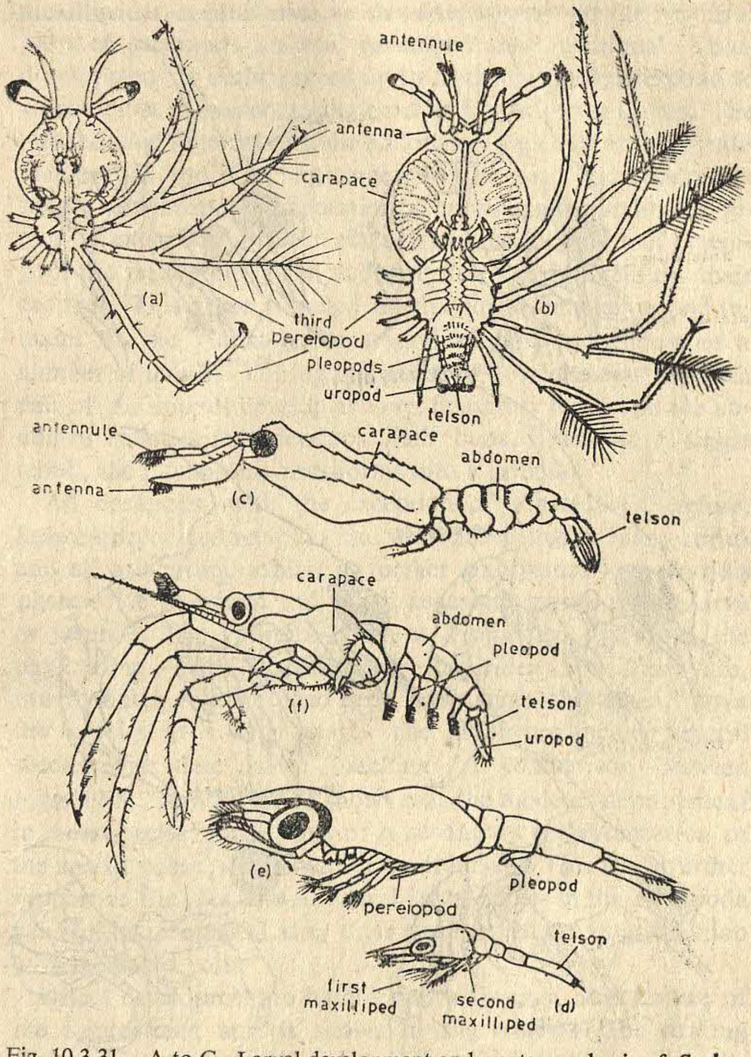


Fig. 10.3.31. A to C—Larval development and metamorphosis of ***Scyllarus***.

A—Stage 1 phyllosoma larva.

B—Stage 7 phyllosoma.

C—Post-larva.

D to F—Larval development and metamorphosis of ***Petrochirus***.

D—Stage 1 zoaea.

E—Stage 5 zoaea.

F—Glaucothoe.

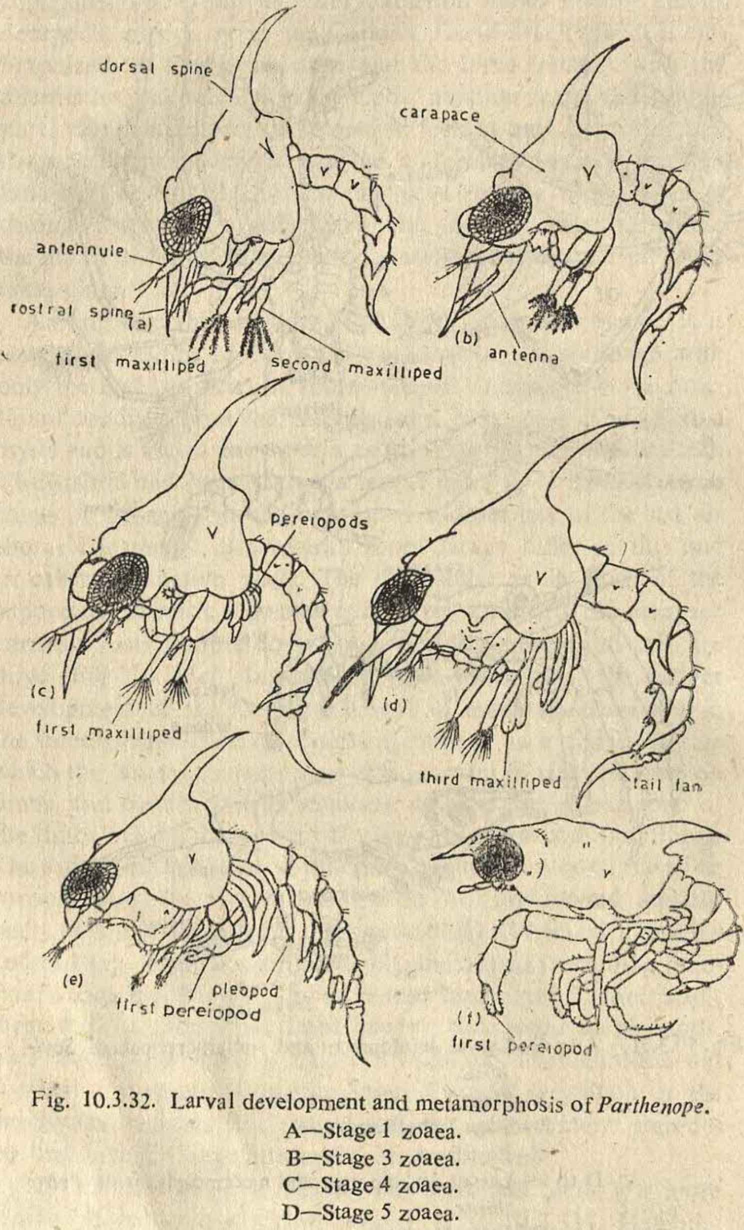


Fig. 10.3.32. Larval development and metamorphosis of *Parthenope*.
A—Stage 1 zoaea.
B—Stage 3 zoaea.
C—Stage 4 zoaea.
D—Stage 5 zoaea.
E—Stage 6 zoaea.
F—Megalopa.

and antenna retain no naupliar features, the mouth parts and maxillipedes approximate to the adult form and the first four pairs of periopods are well developed and functional. Thus, development is embryonized up to a stage which correspond to a post-larva, in respect of the head and most of the thorax. The last thoracic segment and the abdominal segments are still rudimentary at the hatching stage and the head and thorax are highly specialized for floatation. The antennal-mandibular region is expanded as a flat plate, while the four pairs of pereiopods are greatly elongated and carry long setal plumes on their exopods. This stage is called as the phyllosoma larva, and remains in the plankton for several months and undergoes a number of moults. During this phase of development, the fifth pair of pereiopods develop as long, floatatory limbs and the abdomen enlarges and develops limb buds. Then, at a single moult, the phyllosoma transforms into a juvenile.

All decapods, with the exception of penaeidae, therefore, have embryonized nauplius and protozoaea stages. Many carids and all astacurans exhibit a further embryonization of mysis phase of development and hatch as a late mysis, a post-larva or juvenile. The Palinura hatch as a modified post-larva, the phyllosoma. The Thalassinidea, Paguridea and Brachyura, usually hatch as a modified early mysis stage, the zoaea, but a few species at a later stage. The Brachyura include several species with late larval hatching. A comparison between Figs. 10.3.32 and 10.3.33 shows that the mode of development in these species is indicative of a secondary embryonization of the zoaeal phase of brachyuran development. This lends further support to the idea that ancestral larval stages in the Decapoda can still be recognized after their embryonization in association with increased yolk.

Direct development to hatching as a juvenile is a feature of the Leptostraca and is shown in Fig. 10.3.34. The striking feature of these malacostrucans is the serial development of the post-naupliar region. The embryonized nauplius remains distinct, but there is no indication of the embryonization of later larval stages in the development of the post-naupliar region. Two interpretations are possible:

(1) All traces of ancestral larval stages other than the nauplius have been lost, (2) the malacostracan ancestors of the

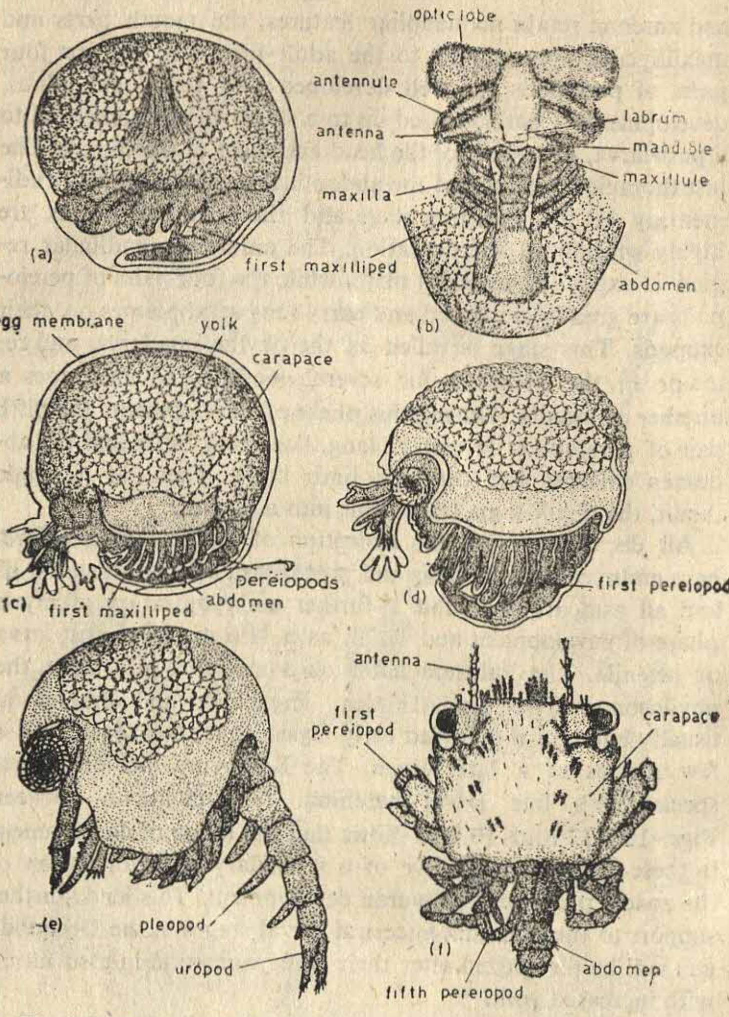


Fig. 10.3.33. External development of the embryo of *Pilumnus*.
A & B—Metanauplius stage in lateral and ventral view.
C—Second embryonic zoaeal stage.
D—Third embryonic zoaeal stage.
E—Fourth embryonic zoaeal stage.
F—Newly hatched megalopa.

Leptostraca, Syncarida and Percarida did not have specialized larval stages other than the nauplius. The second interpretation is more plausible. They probably had a serial development of

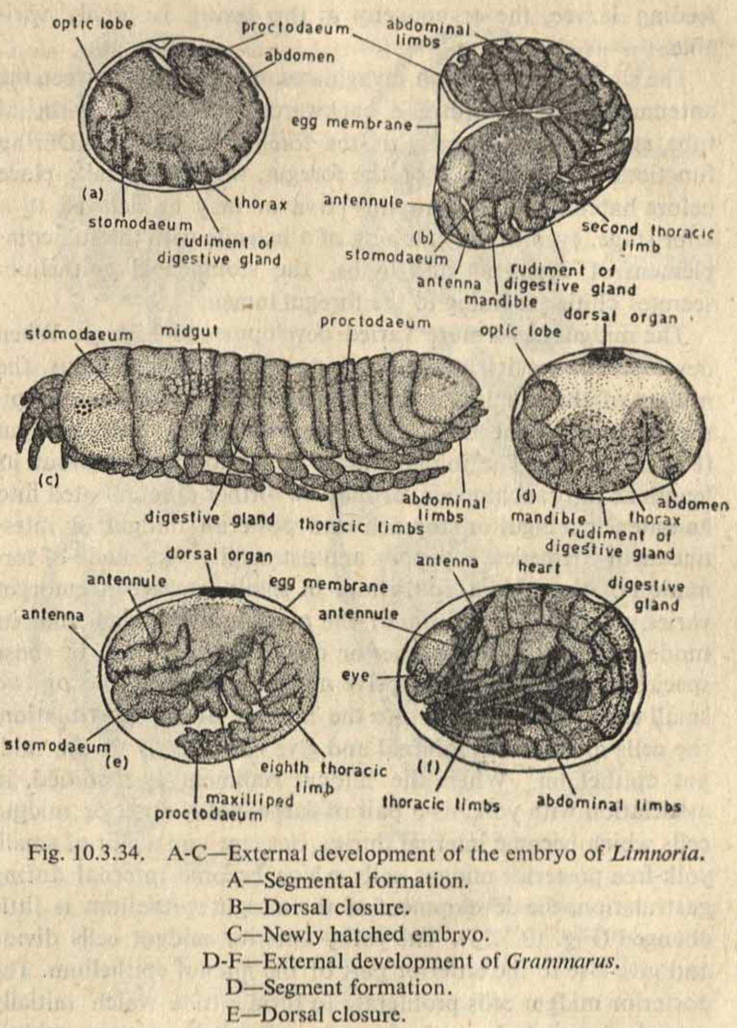


Fig. 10.3.34. A-C—External development of the embryo of *Limnoria*.
A—Segmental formation.
B—Dorsal closure.
C—Newly hatched embryo.
D-F—External development of *Grammarus*.
D—Segment formation.
E—Dorsal closure.
F—Fully developed embryo.

the post-naupliar segments in much the same generalized manner.

FURTHER DEVELOPMENT OF GUT

In view of the great range of modes of development in Crustacea, including adaptation to yolk and specializations as feeding larvae, the organogeny in this group is highly variable.

The stomodaeum, which invaginates midventrally between the antennae, grows inwards and backwards as a simple epithelial tube and develops directly as the foregut epithelium. During functional differentiation of the foregut, which may take place before hatching of the nauplius larva or may be delayed to a later stage, even to the hatching of a juvenile with the full complement of segments and limbs, the stomodaeal epithelium secretes chitinous lining to the foregut lumen.

The midgut has a more varied developmental history. When development results in the hatching of a feeding nauplius, the midgut of the nauplius is functionally differentiated as an epithelial tube connecting the foregut to the short hindgut (Fig. 10.3.27). The midgut may be uniform throughout its length as in branchiopods, or may be further differentiated into an anterior midgut or stomach and posterior midgut or intestine as in cirripedes, copepods and ostracods. The mode of formation of the midgut epithelium in small crustacean embryos varies with the composition of the presumptive midgut and its mode of entry into the interior during gastrulation. In those species in which the presumptive midgut comprises one or two small cells which migrate into the interior during gastrulation, the cells divide when internal and give rise directly to the midgut epithelium. When the midgut rudiment is modified, in association with yolk, as a pair of large, yolky anterior midgut cells which become internal during cleavage and a pair of small, yolk-free posterior midgut cells which become internal during gastrulation, the development of the midgut epithelium is little changed (Fig. 10.3.35). The yolky anterior midgut cells divide and give rise to the anterior part of the midgut epithelium. The posterior midgut cells proliferate to form a tube which initially extends forwards beneath the mass of yolky, anterior midgut cells, but later become connected with the posterior end of the epithelial sac arising from the anterior midgut cells. During its forward growth, the posterior midgut approaches the inner end of the stomodaeum, but the two tubes do not become connected to one another. In cirripedes, with larger eggs, however, in which the anterior midgut cells are large and yolk filled, the posterior midgut tube establish connection with stomodaeum

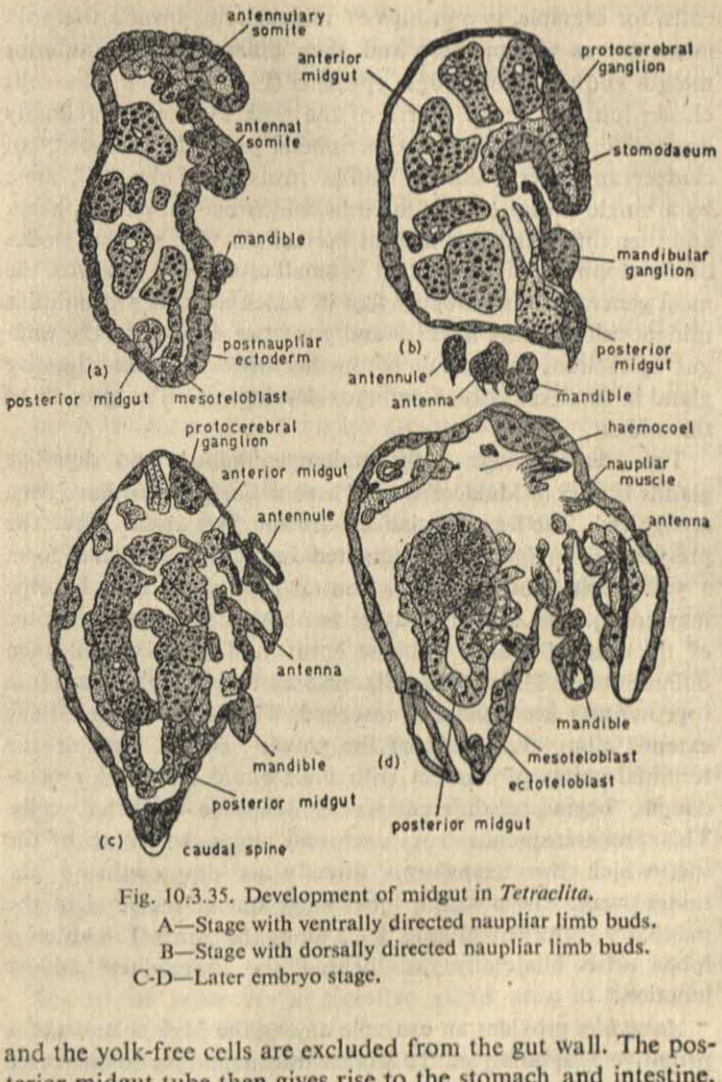


Fig. 10.3.35. Development of midgut in *Tetraclita*.
A—Stage with ventrally directed naupliar limb buds.
B—Stage with dorsally directed naupliar limb buds.
C-D—Later embryo stage.

and the yolk-free cells are excluded from the gut wall. The posterior midgut tube then gives rise to the stomach and intestine, while the yolk cells which lie in the haemocoel are gradually resorbed. This specialization is unusual in Crustacea. The typical midgut adaptation to yolk is a temporary vitellophage action of the midgut cells followed by further development of the same cells as the epithelial cells of the midgut wall. In ostracods, for example, two groups of midgut cells, invade the yolk mass, act as vitellophages and then differentiate as anterior midgut and posterior midgut epithelia (Fig. 10.3.21). The cells cluster initially at the centre of the yolk mass and gradually draw in and digest the more peripheral yolk. The majority of cladoceran embryos show a similar invasion of the yolk mass by a single group of midgut cells which act as vittellophages and then differentiate as midgut epithelium. Of all the modes of development of the midgut in small crustacean embryos, the most generalized is probably that in which a single presumptive midgut cell becomes internal and gives rise directly to the midgut epithelium as a result of further divisions. The digestive gland in small crustacean embryos develops as an outgrowth of the midgut.

The greatest range in the midgut epithelium and digestive glands is seen in Malacostraca. Three major patterns have been recognized. The least specialized are the Syncarida. After the presumptive midgut has invaginated during gastrulation to form a yolky epithelium around a central cavity, the cells become massed together and the cavity is obliterated. At the surface of the mass of cells, a uniform epithelium of yolky cells then differentiates. The central cells enclosed by the epithelium fuse together and are gradually resorbed. The yolky epithelial sac extends along the length of the growing embryo and at the terminal points of contact with the stomodaeum and proctodaeum, begins to differentiate as definitive epithelial cells. This process spreads from each end along the length of the sac, which thus transforms directly as outgrowths of the midgut wall. The posterior lobes bulge out at the level of the maxillary segment and grow back along the trunk. The anterior lobes arise bilaterally just behind the stomodaeal midgut junction.

Anaspides provides an example among the Malacostraca of a primitive adaptation of the malcostracan midgut to increased yolk, functionally analogous to that of clitellates. Some cells are digested, while others act temporarily in the intracellular yolk digestion and then differentiate as the midgut epithelium. In all other examples of malcostracan lecithography the presumptive midgut cells are first cut off as small cells at the surface of the yolk mass. Their subsequent development in relation to yolk digestion and midgut function is therefore more complicated and follows one of two routes.

The pattern of development of the midgut in the Leptostraca, Hoplocarida and many decapod Eucarida follow similar pattern. Cell proliferation from the presumptive midgut area begins with the onset of gastrulation but continues only until the sixth post-naupliar segment has been formed. The cells proliferate early in this process, before the naupliar segments have been individuated at the surface of the embryo, initially form a compact mass between the proliferative area and the surface of the yolk mass. As the formation of the post-naupliar segments proceeds, the proliferative area continues to bud off cells which fill the centre of each segment rudiment, resulting in the formation of midgut strand along the midline of the anterior post-naupliar segments. With cessation of proliferation, the cells of the proliferative area slip into the interior and are added to the posterior end of the midgut strand.

The initial mass of midgut cells, and the strand of cells which forms behind it, behave in different ways during subsequent development. As soon as the initial mass of cells is formed, some of its cells begin to absorb yolk and migrate out in all directions over the surface of yolk mass. Escape of cells from this initial cell mass continues in this way until the cells form a continuous vitellophage epithelium around the yolk mass. This component, the anterior midgut, has a temporary vitellophage function. The midgut strand proliferated along the centre of the anterior post-naupliar segments does not have this function. The strand develops a lumen and differentiates directly as a posterior midgut epithelium. In later development, the outer parts of the cells of the temporary vitellophage epithelium are cut off as definitive epithelial cells of the anterior part of the midgut. The paired lobes of the digestive gland arise as folds of the anterior midgut wall.

In the Decapoda, proliferation of midgut cells from the midgut presumptive area begins precociously, immediately after the completion of the blastoderm formation and also ends early when the formation of the post-naupliar segment is just beginning (Fig. 10.3.22). Most of the proliferated midgut cells invade the yolk mass, absorb yolk and move out to form a peripheral vitellophage epithelium, on the post-ventral surface of the yolk mass. This plate is the posterior midgut rudiment. As post-naupliar segment formation proceeds, the centre of the plate grows out of a posterior midgut tube along the middle of the post-naupliar segments. Numerous cell divisions can be detected in the wall of the tube. Transformation of the vitellophage epithelium into definitive epithelium begins at two paired sites in the decapod embryo, postero-laterally at the junction with the stomodaeum. Both regions bulge out as paired digestive gland rudiments. Epithelial transformation spreads from these sites as the digestive gland grows larger and the yolk mass shrinks, but the central part of the vitellophage epithelium of the anterior midgut is either partly or wholly resorbed.

In general, the development of the crustacean gut shows a variety of adaptations associated with yolk, as a result of which the midgut epithelium is either wholly or partly involved in yolk digestion before becoming the definitive midgut. It seems, that the ancestral crustacean midgut was formed in a simple manner by the repeated division of one or two small, yolky cells. The crustacean stomodaeum and proctodaeum are invaginated as simple epithelial tubes and become connected with the midgut during later embryonic development.

Further Development of Mesoderm

We have already seen that the crustacean presumptive mesoderm moves into the interior during gastrulation and clumps together as a group of cells internal to the post-naupliar ectoderm. From this mesodermal source, paired bands of mesodermal cells are proliferated forwards bilaterally beneath the naupliar embryonic ectoderm. The cells of the naupliar mesodermal bands then become grouped as three pairs of naupliar somites (Fig. 10.3.20), leaving a residual mass of post-naupliar mesoderm at the site of proliferation. In front of the antennulary somites, pre-antennulary mesoderm is formed. The origin of this mesoderm is variable. Within the residual post-naupliar mesoderm, a number of cells enlarge to form mesoteloblasts, bilaterally arranged on either side of the ventral midline. The mesoteleblasts are the source from which all the mesoderm of the post-naupliar segments is proliferated, except for some or perhaps all of the mesoderm cells of the maxillulary and maxillary segments. The remainder of the residual mesoderm, left after differentiation of mesoteloblasts, persists at the posterior end of the growing trunk and gives rise to the telson mesoderm and proctodaeal mesoderm.

Two generalizations can be made about mesodermal development in the Crustacea. Firstly, the mode of formation of the mesodermal somites observed in species with small eggs and naupliar larval development persists with little change in species with larger eggs and embryonized later development. Secondly, the external heteronomy of development of the naupliar and post-naupliar segment in Crustacea is also clear in their mesodermal development and persists even when the precocious functional differentiation of the naupliar mesoderm as larval muscles no longer takes place. These factors create some difficulties in attaining a general understanding of somite development and mesodermal organogeny in the Crustacea, since the development of the naupliar somites is specialized even in its basic condition in species which hatch as nauplii and the origin of the post-naupliar somites is specialized in all the species. On the other hand, in some species, the specialized origin of the post-naupliar somites is followed by a relatively generalized mode of further development of the three somites.

Mesoteloblasts: In Cirripedia there are eight mesoteloblasts in two bilateral groups of four. Each group arises by division of a single mesoteloblast mother cell (Fig. 10.3.20). When the mesoteloblast begins to bud off the mesoderm of the trunk segments, they proliferate a succession of transverse rows of eight cells, bilaterally arranged in transverse half rows of four. Each row of eight cells is the rudiment of a pair of trunk somites.

One of the curiosities of crustacean development is that the same specialization of eight mesoteloblasts in paired, bilateral arcs of four has evolved convergently in the Malacostraca, although the functional significance of eight mesoteloblasts, as opposed to another number is still obscure (Fig. 10.3.36). The origin of the eight mesoteloblasts in the Malacostraca within the post-naupliar mesoderm rudiments varies in different species. The malacostracan mesoteloblasts, like those of cirripedes, bud off successive rows of eight cells, each row being the rudiment of a pair of somites (Fig. 10.3.36).

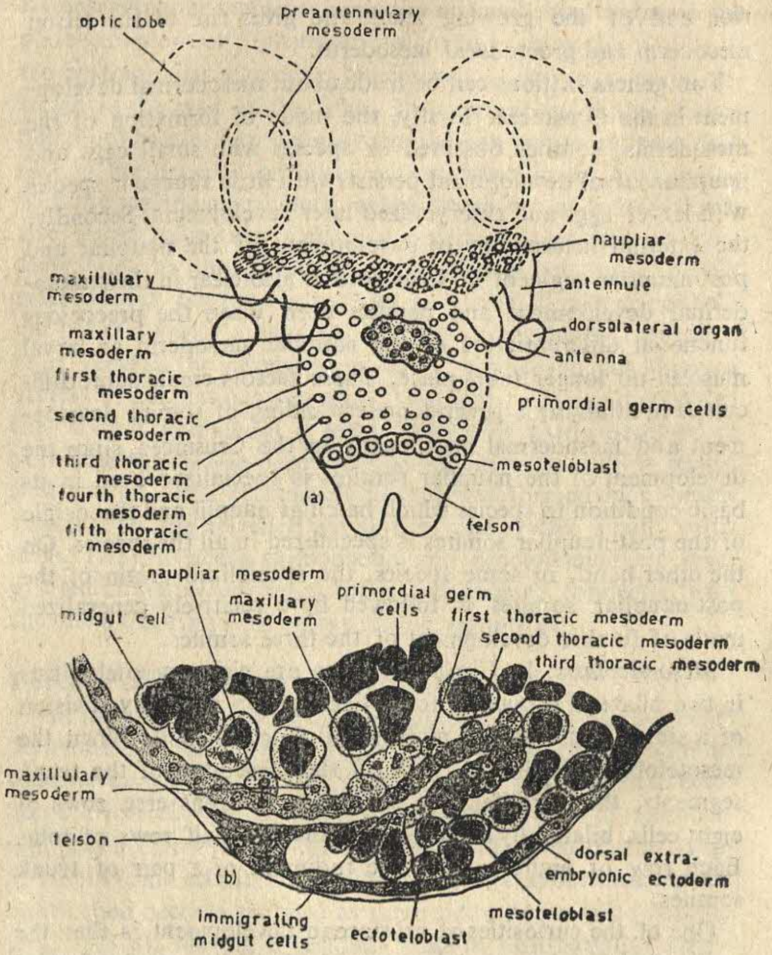


Fig. 10.3.36. Development of the post-naupliar region of the embryo of *Hemimysis*.
A—Diagrammatic ventral view of the germ band early in the growth of the caudal papilla, showing the distribution of mesoderm at this stage.
B—Sagittal section through the forwardly flexed caudal papilla of the same embryo.

Naupliar Somites

The further development of the naupliar somites in the embryos which hatch as nauplii has been studied mainly in the

Cirripedia and the anostracan Branchiopoda. The naupliar somites of the cirripede embryo give rise to the musculature of the three naupliar segments, including the intrinsic and extrinsic muscles of the naupliar limbs (Figs. 10.3.20 and 10.3.35). Prior to their dispersion and differentiation as musculature, the somites are solid blocks of cells, which extend an appendicular lobe into the corresponding limb bud, but do not develop coelomic cavities or participate in the formation of the heart or pericardial septum. The latter structures are not present in the cirripede nauplius. A thin layer of circular splanchnic muscle covers the external surface of the midgut of the cirripede nauplius, but its origin as splanchnic mesoderm has not yet been established. The extrinsic muscles of the three pairs of naupliar limbs comprise a series of dorsal muscles attached to the carapace of the larva and a series of ventral muscles attached to ventral, skeletal endosternites. The dorsal muscle consist of four pairs to the antennules, ten pairs to the antennae, and five pairs to the mandibles. The ventral muscle consists of one antennulary pair, six antennal pairs, and three mandibular pairs. In addition, there are muscles which run between the dorsal carapace and ventral endosternites and others which run between the endosternites themselves.

The development of the naupliar somites in large crustacean embryos, in which the naupliar region is embryonized, continue to reflect their ancestry as components of a functional nauplius. Coelomic cavities are not formed and the major product of somite differentiation is the somatic musculature of the three segments, antennulary, antennal and mandibular. The changes which take place in the musculature of these three segments between the stage of their first functional differentiation and the stage of completion of the adult configuration of the musculature are little understood.

Mesodermal Aspects of Cirripede Metamorphosis

During this process, the naupliar limb muscles and labral muscles are all histolysed and their fragments are digested by phagocytic haemocytes. The antennal and mandibular segments become vestigial and do not redevelop a functional musculature in the cypris stage, but the antennulary segment develops a new set of ten pairs of antennulary extrinsic limb muscles, entirely different in their functional configuration from those of the nauplius. Other muscles also develop in the naupliar region, in association with the formation of the bivalve carapace. The functional transition is achieved through the formation of the cypris muscles as rudiments in the stage VI nauplius, while the naupliar musculature is still intact, and by a rapid histolysis of naupliar muscles and simultaneous onset of function of the cypris muscles at the nauplius-cypris moult. The onset of function of the trunk segments in cirripedes also coincides with metamorphosis from the stage VI nauplius to the cypris. The onset of maxillulary and maxillary function is even further delayed, until the cypris has settled. In the trunk segments, the musculature of the six pairs of trunk limbs is formed from the segmental somites in the usual way, although these somites do not give rise to heart, pericardial floor or gonad components. The limb musculature of the trunk differentiates mainly in the stage VI nauplius at the same time as the antennulary and carapace muscles of the cypris, but does not become functional until the nauplius-cypris moult has taken place. There is no evidence that the trunk somites contribute to the splanchnic mesoderm of the cirripede gut in the usual crustacean manner. The splanchnic musculature formed during embryonic development, before the trunk somites have been proliferated, appear to persist in a modified form after metamorphosis.

Pre-antennulary Mesoderm

Whether the mesodermal components anterior to those derived from the antennular somites should be assigned a segmental origin in the Crustacea is a question of long standing. The evidence for the presence of a pair of pre-antennulary somites in the embryos by species which hatch as nauplii is very limited. Cirripede embryos do not have these somites. Their pre-antennulary mesoderm, derived from the anterior ends of the mesodermal bands, differentiates into labral and stomodaeal musculature and shows no trace of the pattern of differentiation characteristic of the naupliar somites.

Preantennulary mesoderm in the Malacostraca gives rise to the labral and stomodaeal musculature, but in addition gives rise to the anterior aorta above the stomodaeum.

SEGMENTAL ORGANS

In addition to the coelomoduct segmental organs known to specialize as excretory organs in the antennal and maxillary segments, it now seems possible, that vestigial segmental organs develop in a number of other segments. The gonoducts of Crustacea are also of coelomoduct origin.

A pair of antennal segmental organs develops in the embryo of all crustaceans and becomes functional as larval excretory organs in those species which hatch as nauplii. In the majority of the groups, the antennal segmental organs are transitory and are replaced by the maxillary segmental organs as development continues. The development of the antennal segmental organs from the cells of the antennal somites has been described for several species. The gland usually develops from two groups of antennal mesoderm cells. One group hollows out and gives rise to the end sac, while the other group of cells forms the duct. Only the short, terminal exit duct is formed by ectoderm. The maxillary segmental gland usually develops from two components derived from the mesoderm of the maxillary somite, a dorsal component which forms the end sac and a lateral component which forms the coiled duct. The ducts open on the maxilla by a short, ectodermal exit duct. The fact that crustacean antennal and maxillary glands are coelomoduct segmental organs is indisputable. The tentative identifications of vestigial segmental organs in other segments in several crustacean embryos are not so well-founded.

Gonads and Gonoducts

The development of the gonads in the Crustacea always begins with the differentiation of primordial germ cells, but the site of origin of these cells and the timings of their first visible differentiations is highly variable among species. In the malacostracans a pair of germ cells differentiates in the ventral walls of each pair of segmental coelomic cavities in the pericardial septum. The germ cells proliferate to form small groups of cells, which link up as paired strands suspended from the undersurface of the pericardial floor. The segmental coelomic cavities in the pericardial floor are obliterated during this process. Each strand of germ cells is invested by mesodermal cells derived from the pericardial floor and hollows out by internal splitting. The cavity of the gonad is thus formed independently of the vestigeal coelomic cavities. In many malacostracans the gonad rudiments are more compact and temporarily occupy the median faces of the maxillary or first trunk somites before they extend back beneath the pericardial floor. During the proliferation of the post-naupliar rudiment, the germ cells remain associated with the mesoteloblasts until the mesoderm of the first pair of thoracic somites is formed. They then attach in two bilateral groups to the median faces of the first thoracic somites and are carried upwards to a dorsolateral position beneath the pericardial floor as the somites continue their development.

The precocious differentiation of a single primordial germ cell in the wall of the blastula has been observed in several small-egged crustaceans (Fig. 10.3.18).

The variations in the mode of development of the gonads in the Crustacea preclude the formation of a generalized statement of this topic. It is clear that the primordial germ cells are closely associated with the mesoderm, but vary in their time of first visible differentiation. The cavities of the gonads develop independently of the segmental coelomic cavities, but the gonoducts are coelomoducts of a variable genital segment.

Further Development of the Ectodermis

One of the most useful results of comparison of fate maps in the Crustacea (Fig. 10.3.18) is the recognition of a stable pattern of sub-areas within the presumptive ectoderm. This pattern is of interest in two ways. Firstly it shows that the basic ectodermal components of the nauplius are already established at the blastula or blastoderm stage. There is a median protocerebral area anteriorly, paired antennulary, antennal and mandibular area posteriorly. The further development of the ectoderm can be discussed in terms of these areas, whether the outcome at the hatching stage is a nauplius larva or a fully formed juvenile. Secondly, the pattern displays a dorsal area of presumptive ectoderm which, in some small embryos, in presumptive carapace ectoderm, but in larger embryos is modified as presumptive extra-embryonic ectoderm enclosing the large yolk mass. In later development, the extra-embryonic ectoderm partially reverts to a definitive fate as dorsal ectoderm and partially undergoes resorption in various ways.

In general, the further development of the naupliar ectoderm proceeds in a relatively simple manner, yielding the epidermis and epidermal glands, the ganglia of the brain and ventral cord, the ventral exoskeletal invaginations to which many of the somatic muscles are attached, and the short, ectodermal exit ducts of the antennal and maxillary segmental organs and gonaducts. The major event which must proceed much of the differentiation is the proliferation of the ectoderm of the post-naupliar ectoderm. The proliferation may occur during larval development, after hatching as nauplius, or may be completed to 7 differing extents before hatching takes place. Present evidence shows that whatever the degree of post-naupliar segment formation before or after hatching, the ectoderm of these segments is proliferated by ectoteloblasts.

Ectoteloblasts

The ectoteloblast cells originate by cell enlargement in a transverse band, sometimes curved posteriorly at its ends (Fig. 10.3.24), within the post-naupliar presumptive ectoderm. Anterior to the ectoteloblast this ectoderm contributes to a greater or lesser extent to the ectoderm of the maxillulary and maxillary segments, but there seems no doubt that all of the ectoderm of the trunk segments is of ectoteloblast origin. The post-naupliar presumptive ectoderm posterior to the ectoteloblasts gives rise to the ectoderm of the telson. The ectoteloblasts of malacostracan embryos bud off successive transverse rows of ectoderm cells in a forward direction (Figs. 10.3.19 and 10.3.36). With further division of these cells, the ectoderm rows become a generalized trunk ectoderm, which subsequently becomes segmentally delineated in association with the paired mesodermal teloblasts. The ectoteloblast row of malacostracans curves towards the midline at its free ends during early budding, so that it becomes a ring of cells. The further products of this ring then form a tubular contribution of the trunk, further forwards beneath the ventral surface of the yolk filled anterior part of the embryo (Figs. 10.3.30, 10.3.34 and 10.3.36). When the ectoderm of the last abdominal segment is formed, the ectoblasts can no longer be distinguished.

Nervous System

The ganglia of the central nervous system in the Crustacea arises by the inward proliferation of numerous small cells from the ectoderm. The basic components of the nervous system are a pair of relatively large protocerebral ganglia developed from the protocerebral ectoderm, paired antennulary and antennal ganglia developed from the ventrolateral presumptive ectoderm segments, and a paired, mid-ventral ganglion formed from the ventral ectoderm of each postnaupliar segment (Figs. 10.3.21 and 10.3.37). All of the ganglia separate into the interior, leaving epidermis at the surface. The antennulary or deutocerebral ganglia and the antennal or tritocerebral ganglia make up paired areas of nervous tissue which pass on either side of the stomodaeum and link two protocerebral ganglia with the mandibular ganglion (Fig. 10.3.27). The tritocerebral commissure is postoral. In embryos which hatch as nauplii the anterior part of the nervous system from the protocerebral ganglia to the mandibular ganglion, is fully developed and functional (Figs. 10.3.21, 10.3.27 and 10.3.35), though it undergoes further changes as larval development proceeds. The ganglia of the maxillulary, maxillary and trunk segments are then developed as part of the elaboration of their respective segments. When naupliar development is embryonized and hatching occurs at a later stage, the distinction between the development of the naupliar and post-naupliar parts of the nervous system is less marked, but still persists as a residual aspect of heteronomous development. The nauplius eye and the compound eyes are developed from the protocerebral ectoderm in conjunction with the cerebral ganglia. The only question which is not fully resolved on the basic composition of the crustacean nervous system is the existence of a pair of pre-antennulary segmental ganglia distinct from the main protocerebral ganglia. A pair of small ganglia has been identified during the development of the brain of several species, lying between the protocerebral ganglia and the antennulary ganglia. In view of the absence of corresponding limb buds and the difficulties of interpreting the pre-antennulary mesoderm as a pair of segmental somites, the segmental significance of the pre-antennulary ganglia remains undecided.

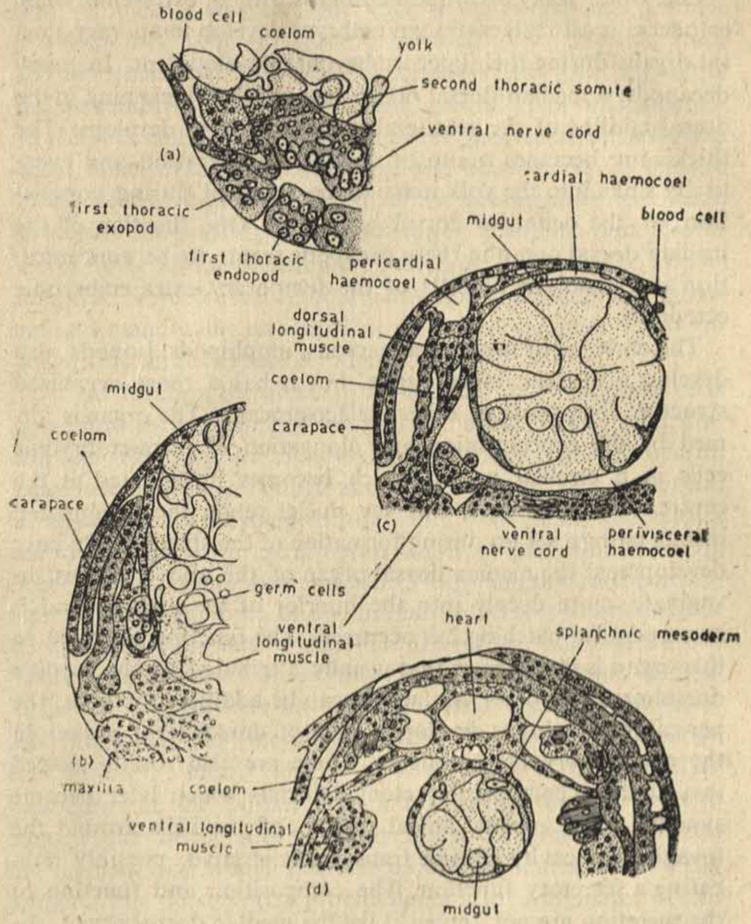


Fig. 10.3.37. Development of post-naupliar mesoderm of *Hemimysis*.

A—2nd thoracic segment, showing early development of segmental somite.

B—1st thoracic segment showing dorsal upgrowth and further differentiation of somite.

C—2nd thoracic segment, showing formation of the heart.

D—6th thoracic segment after completion of the heart at this level.

Dorsal Organs

Like other yolky arthropod embryos with an extra-embryonic ectoderm most malacostracan embryos develop temporary dorsal organs during their later embryonic development. In many decapods, a median dorsal organ as a cellular thickening in the dorsal midline of the extra-embryonic ectoderm develops. The thickening becomes a site of cellular degeneration and eventually sinks into the yolk mass to be resorbed during completion of the definitive dorsal body wall. One function of the median dorsal organ in these decapods seems to be concentration and histolysis of cells of the temporary extra-embryonic ectoderm.

The embryos of mysids, cumaceans, amphipods, isopods also develop a median dorsal organ, but it has a more organized structure than those of other malacostracans. The organ is formed by the concentration and elongation of extra-embryonic cells in a circular patch, which becomes invaginated at the centre. The cells fuse, but the nuclei retain the invaginated rosette pattern set up during formation of the structure. In later development the median dorsal organ of the above groups invaginate more deeply into the interior of the embryo and is resorbed after hatching has occurred. The function ascribed to this organ is the same as that usually attributed to the median dorsal organ of other Malacostraca. In addition to these, the pericardean embryos develop a pair of dorsolateral organs in the extra-embryonic ectoderm. These are also rosette shaped invaginations composed of elongated cells which later become syncitial. The narrow apical regions of the cells around the invagination cavity become transversely striated, possibly indicating a secretory function. The composition and function of the secretion are not known. Like the median dorsal organ, the dorsolateral organs eventually sink beneath the surface of the embryo and are resorbed.

The Basic Pattern of Development in Crustacea

Comparative studies shows that the basic pattern of development in the Crustacea include a small yolky egg, a modified spiral cleavage, hatching as a nauplius larva and anamorphic development of the post-naupliar segment during a series of larval stages with intervening moults.

The basic crustacean eggs can probably be envisaged as having a diameter of 100-200 μ. Cleavage is spiral, but is modified in such a way that the yolk is segregated into a single posteroventral cell, 4P, partially covered at the blastula stage by the remaining, yolk-free blastomeres. The yolk cell 4D is the presumptive midgut. The three ventral stem cells anterior to the yolk cell, 3A, 3B and 3C are the presumptive mesoderm cells. Some of the descendants of the blastomeres 2b and 3b in the ventral midline in front of the presumptive mesoderm constitute the presumptive stomodaeum. The remaining blastomeres make up the presumptive ectoderm already zoned as presumptive protocerebral ectoderm anteriorly, antennulary, antennal and mandibular ectoderm laterally, post-naupliar ectoderm posteriorly and carapace ectoderm dorsally.

At gastrulation the presumptive midgut and mesoderm cells move into the interior and are replaced at the surface by a ventral spreading of the mandibular and post-naupliar ectoderm. Once internal, the yolky presumptive midgut cell divides several times and forms the midgut epithelium, functionally differentiated as the midgut of the nauplius larva by the time hatching occurs. The presumptive mesoderm cells migrate to the posterior end of the embryo to lie internal to the post-naupliar ectoderm and proliferate a pair of lateral mesodermal bands. These grow forwards on either side of the developing midgut as far as the protocerebral ectoderm, and become subdivided into pre-antennulary mesoderm anteriorly, three pairs of naupliar somites laterally and a residual mass of post-naupliar mesoderm at the posterior end. Meanwhile the presumptive stomodaeum invaginates mid-ventrally at the level of the antennal segment and becomes connected with the anterior end of the midgut. The presumptive proctodaeum also invaginates at the posterior midpoint of the post-naupliar ectoderm and becomes connected with the posterior end of the midgut, thus completing the functional gut of the nauplius. As it grows inwards the proctodaeum pushes through the residual, post-naupliar mesoderm. In the further development of the mesoderm, the pre-antennulary mesoderm gives rise to the labral and other musculature at the anterior end of the nauplius and to the stomodaeal musculature. It is possible that the pre-antennulary mesoderm includes the vestiges of a pair of pre-antennulary somites. The naupliar somites gives rise to the complex musculature of the three pairs of naupliar limbs and perhaps contribute a thin layer of splanchnic mesoderm on the external surface of the midgut. A pair of antennal glands (coelomoduct segmental organs) is also formed from cells of the antennal somites. The post-naupliar mesoderm develops into four main components, the maxillulary and maxillary somites, a bilateral ring of mesoteloblasts behind these somites, and the mesoderm of the telson and proctodaeum. The mesoteloblasts may bud off the somite rudiments of one or more post-maxillary segments before hatching takes place, but none of the post-naupliar segments is functional in the newly hatched nauplius.

In the further development of the ectoderm the general product is the ectoderm of the nauplius. The protocerebral ectoderm also gives rise to the protocerebral ganglia, possibly including a pair of small ganglia of pre-antennulary segment and to the median naupliar eye. The ventral protocerebral ectoderm pouches out as labrum with ectodermal labral glands. The naupliar segmental ectoderm pouches out ventro-laterally as a pair of limb buds in each segment, the antennules being pre-oral, the antennae para-oral and the mandibles post-oral. The antennules remain uniramous, but the antennae and the mandibles become biramous. Each pair of naupliar limbs develops to a functional condition, with the long rami and, on the antennae and mandibles, basal ingestive setae. The ventral ectoderm of the naupliar segments gives rise to paired segmental ganglia. A pre-oral, antennulary pair are attached to the protocerebral ganglia. The antennal ganglia are para-oral, with a post-oral commissure, and the mandibular ganglia occupy a median, ventral position behind the mouth. The post-naupliar ectoderm develops as four components which correspond to those of the mesoderm. These comprise a ring of maxillulary ectoderm, a ring of maxillary ectoderm, a ring of ectoteloblasts and a posterior cap of telson ectoderm with the anus at its midpoint. The ectoderm of one or more post-maxillary segments may be budded from the ectoteloblasts before hatching takes place, but none of the post-naupliar segments has functional limbs in the newly hatched nauplius.

In the generalized conditions, there is little change in the fundamental structure of the naupliar region during larval development and metamorphosis, except for some changes in the mandibular musculature associated with the loss of the naupliar ingestive setae and onset of function of the mandibular gnathobases at metamorphosis, and for the development of the compound eye as outgrowths of the protocerebrum. The development of the post-naupliar region, in contrast, procceds in antero-posterior succession, with metamorphosis from the nauplius to the juvenile coinciding with the completion of six pairs of functional trunk limbs. The first pair of post-naupliar limbs, the maxillules, is cephalized before metamorphosis takes place, indicating that the maxillary segment is part of the generalized crustacean head. The second pair of post-naupliar limbs, the maxillae, is functionally the first pair of trunk limbs in its most generalized condition, indicating that the maxillary segment is a later addition to the crustacean head. The question of the number of segments in the generalized crustacean head, however, is irrelevant.

The post-naupliar segments are proliferated serially from the teloblastic growth zone in front of the telson and are built around the elongating midgut. The mesoteloblasts proliferate continuations of the lateral mesodermal bands which divide antero-posteriorly into bilateral arcs of cells, the somites, lying against the ectoderm. The ectoteloblasts proliferate a generalized cylindrical ectoderm which become segmentally delineated in association with the paired somites.

When first formed, the somites are separated from the midgut by a wide haemocoel. Each segment then develops directly to the juvenile adult condition, with no temporary larval specializations. The segmental ectoderm pouches out ventro-laterally as limb buds and gives rise mid-ventrally to the paired ganglia of the segment. Invaginations of ectoderm form endophragmal skeletal bars and also form the short exit ducts of the maxillary segmental organs and the gonoducts. The gonopores lie in the middle region of the trunk.

The paired somites first produce median extensions across the haemocoel to meet the midgut dorsolaterally, forming the pericardial floor. A pair of vestigial coelomic cavities then develop in the pericardial floor and the somites become elaborated as heart middorsally, dorsal longitudinal muscle dorsolaterally in the pericardial haemocoel, and limb musculature and ventral longitudinal muscle ventrolaterally. The splanchnic mesoderm at each segmental level is formed by a downward growth of mesoderm over the midgut from the median edge of the pericardial floor. It is notable that the three pairs of naupliar somites are specialized and retain only certain aspects of this generalized pattern. They do not contribute to the heart, pericardial floor or longitudinal muscles.

Among the post-naupliar somites, only the maxillary pair give rise to a pair of coelomoduct segmental organs, the maxillary glands, but there is evidence to suggest that segmental organs were a feature of all trunk somites in ancestral crustaceans. The gonoducts are coelomoducts, but the development of the gonads is not closely associated with the vestigial coelomic cavities. The primordial germ cells temporarily occupy the ventral walls of these cavities, at least in the interior part of the trunk, but as the germ cells proliferate and join up to form paired strands, the strands move down to become depended from the ventral surface of the pericardial floor. Each strand, which is invested by mesoderm cells from the pericardial floor, then hollows out to form a cylindrical gonad. The gonocoels are thus developed independently of segmental coelomic cavities, though they later gain communication posteriorly with the coelomoduct gonoducts. It is not certain whether, in the generalized condition, the primordial germ cells of Crustacea show their first visible differentiation in the somites or are differentiated precociously and migrate into the somites secondarily.

The Crustacea thus exhibit a distinctive theme of generalized development, quite different from those of other arthropods. The theme persists in all extant species in spite of manifold and extreme variations and provides strong evidence of the monophyletic nature of the Crustacea as a group.

4. APTERYGOTA

Eggs

All apterygote eggs have a central nucleus in a small cytoplasmic halo, surrounded by uniformly distributed yolk granules in a sparse cytoplasmic reticulum. A few workers have reported the presence of a thin layer of yolk-free cytoplasm, the periplasm at the surface of the egg. The egg is enclosed in an external chorion secreted by the follicle cells of the parent ovariole and a few of them are also said to have a thin vitelline membrane beneath the chorion.

Cleavage

Marked differences are displayed between classes in the size, shape and mode of cleavage of the egg. The thysanuran eggs are ovoid, about 1.0 mm long and 0.8 mm in diameter. Cleavage is intralecithal (Fig. 10.4.1) with repeated mitosis, leading to the formation of numerous cleavage energids within the yolk mass. The first three cleavage divisions in *Lepisma* are synchronous,

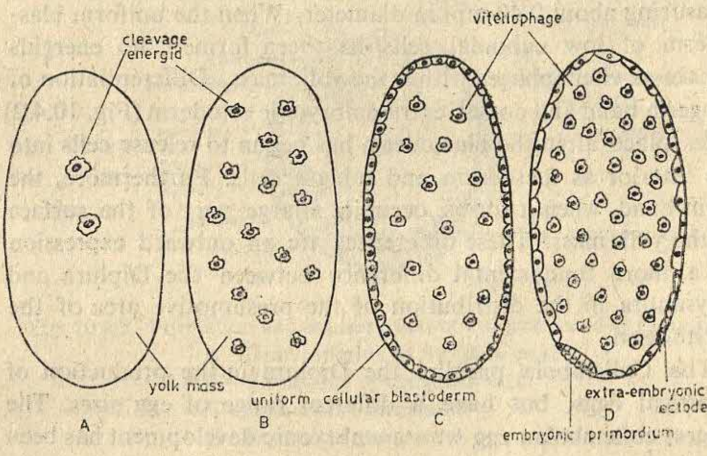


Fig. 10.4.1. Cleavage in the Thysanura.
A—Early interlecithal cleavage.
B—Later interlecithal cleavage.
C—Uniform blastoderm.
D—Differentiated blastoderm.

but thereafter synchrony is lost. The energids spread through the yolk mass and many of them emerge at the surface and divide further to form a uniform blastoderm of low cuboidal cells. Other energids persist within the yolk mass and assume the role of vitellophages. The uniform blastoderm then differentiates, through a differential concentration of cell division towards the posterior end and gradual aggregation of most of the cells into a small posterior or postero-ventral disc, to form a compact embryonic primordium and attenuated dorsal, extra-embryonic ectoderm. The cells of the embryonic primordium are columnar and closely packed. The extra-embryonic ectoderm consists of the usual flattened cells spread out as a thin layer over most of the surface of the yolk mass.

The differentiation of the uniform blastoderm in this way to form a compact embryonic primordium is confined to Thysanura among the apterygotes and is otherwise seen only in the pterygote insects.

The group Diplura, e.g. *Campodea*, has spherical eggs, measuring about 0.40 mm in diameter. When the uniform blastoderm of low cuboidal cells has been formed, no energids remain as vitellophages within the yolk mass. Differentiation of the germ band and dorsal, extra-embryonic ectoderm (Fig. 10.4.2) takes place after the blastoderm has begun to release cells into the interior as mesoderm and midgut cells. Furthermore, the germ band, when it forms, occupies a large part of the surface of the yolk mass. These differences are an outward expression of a more fundamental difference between the Diplura and Thysanura in the distribution of the presumptive area of the blastoderm.

The Collembola parallel the Diplura in the production of spherical eggs, but have a different range of egg sizes. The largest collembolan egg whose embryonic development has been studied is that of *Tetradontophora*, with a diameter of 0.5 mm. Throughout the entire range of size, collembolan eggs exhibit a phase of total cleavage(Figs. 10.4.3 and 10.4.4) preliminary to the formation of a blastoderm. The first three divisions are synchronous. After the eight-cell stage, cleavage divisions continue to be equal but become irregular in timing and distribution, yielding a sphere of pyramidal, yolky blastomeres around a small central cavity. As the blastomeres become narrow, the

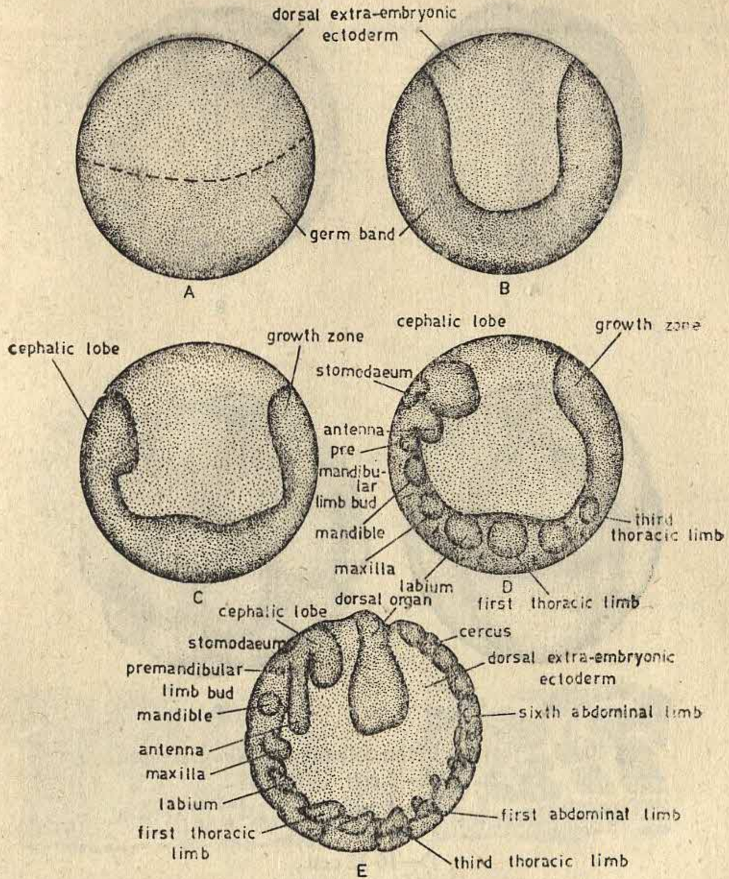


Fig. 10.4.2. Formation and segmentation of the germ band of *Campodea*.
A—Differentiation of the germ band.
B & C—Elongation of the germ band.
D—Segmenting germ band.
E—Segmented germ band.

nucleus and associated cytoplasmic halo within each blastomere moves out towards the exposed surface of the cell. By the time 32-blastomeres have been formed, the nuclei reach this surface. The innermost parts of the pyramidal blastomeres are cut off as anucleate yolk masses during these division and the succeeding divisions which lead to the 64-cell stage. The yolk masses fuse into a single mass, filling the interior of the embryo, leaving a

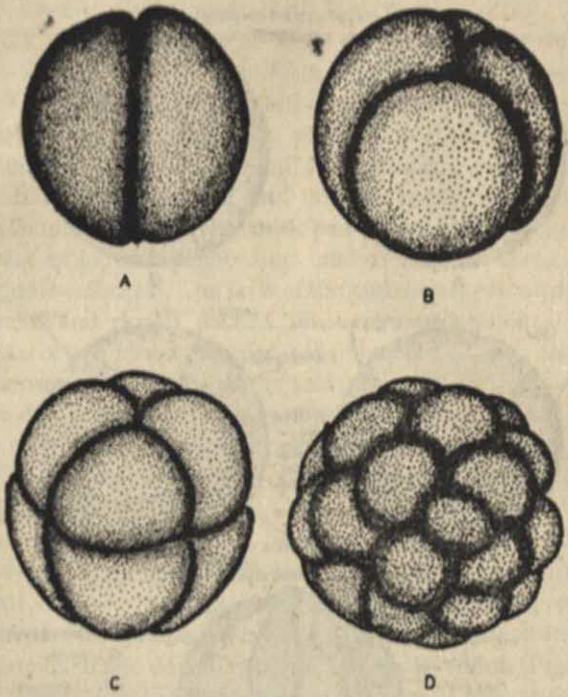


Fig. 10.4.3. Cleavage in the collembolan *Anurida maritima*.
A—2 cell.
B—4 cell.
C—8 cell.
D—16-32 cell.

layer of truncated pyramidal blastomeres at the surface. At about 64-cells radial divisions ensure in the pyramidal blastomeres, cutting off yolky, polygonal cells inwards and leaving cuboidal, yolk-free cells at the surface. The peripheral cells continue to divide with tangential spindles and give rise to the uniform blastoderm. The first two to five cells cut off into the interior migrate to the centre of the embryo, where they undergo further divisions to form a compact mass of polygonal cells. The remaining internal cells beneath the blastoderm unite with each other to re-establish a unitary yolk mass containing vitellophages. The polygonal cells at the centre of the yolk mass are primordial germ cells.

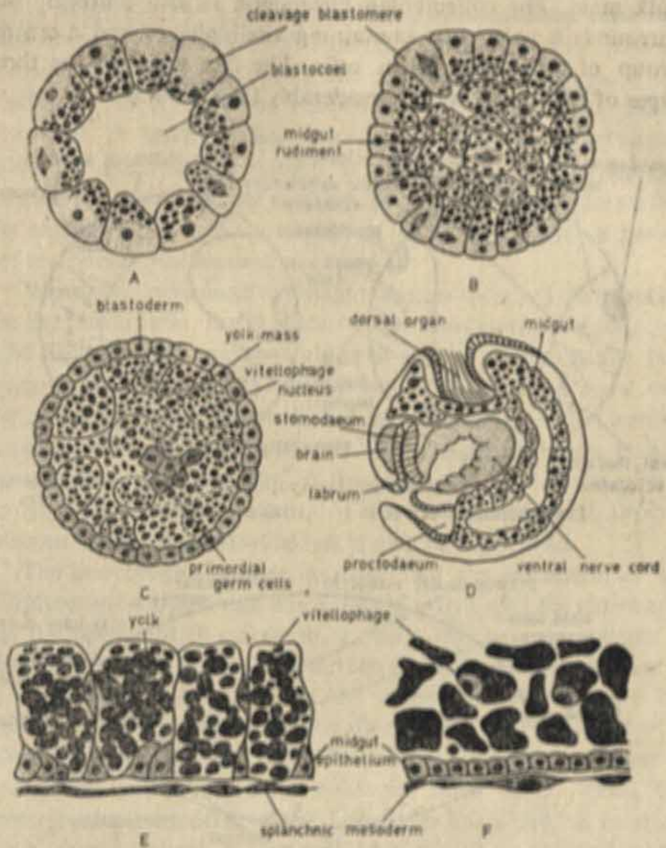


Fig. 10.4.4. *Tetradontophora bielanousis.*
A—Section through late stage of total cleavage.
B—Section showing blastoderm formation.
C—Section through blastoderm stage.
D—Sagittal section through flexed embryo.
E—T.S. through early midgut wall.
F—T.S. through late midgut wall.

The thysanuran blastoderm, becomes differentiated into a small, posterior embryonic primordium and an attenuated extra-embryonic ectoderm and surrounds a yolk mass whose vitellophages are persistent cleavage energids. The dipluran blastoderm is a uniform layer of cuboidal cells around an anucleate yolk mass. The collembolan blastoderm is also uniform, but surrounds a yolk mass containing vitellophages and a central group of primordial germ cells. The fate maps of the three types of blastoderm vary considerably (Fig. 10.4.5).

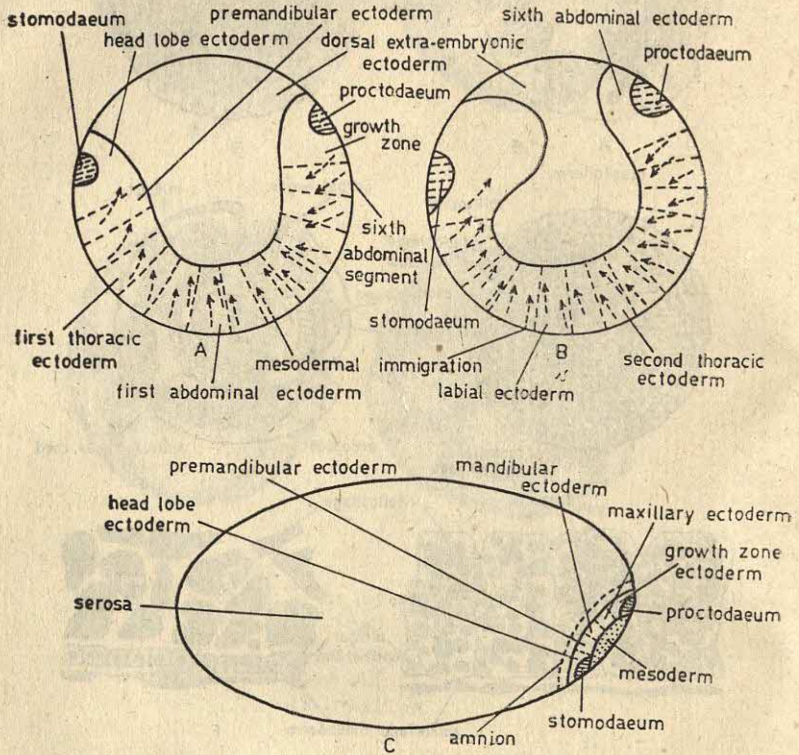


Fig. 10.4.5. Fate maps of the apterygote blastoderm.
A—Diplura.
B—Collembola.
C—Thysanura.

The presumptive midgut of the Thysanura is known to be the vitellophages left within the yolk mass during cleavage and formation of the blastoderm and lacks blastodermal representation. The blastoderm of the dipluran, *Campodea*, releases scattered cells into the yolk mass and these cells, after acting as vitellophages, subsequently give rise to the midgut epithelium. If this is correct, the presumptive midgut has no localized representation in the blastoderm, and retains a gastrulation movement only in the form of a diffuse migration of blastoderm cells into the interior. The collembolan midgut epithelium also develops from the vitellophages within and around the yolk mass. These cells are cut off into the interior by radial divisions during cleavage. A generalization can thus be made for apterygotes, that the presumptive midgut is constituted by vitellophages which attain an internal position during blastoderm formation or earlier in cleavage and persist as midgut cells, after a period of temporary vitellophage activity.

When the presumptive midgut has no localized distribution in the blastoderm, the locations of the presumptive stomodaeum and proctodaeum can be established only in relation to the presumptive ectoderm. The vents of latter development show that in all apterygotes, the stomodaeum invaginates in the ventral midline between the head lobes and the proctodaeum invaginates at the posterior end of the germband, behind the growth zone. The presumptive areas of the stomodaeum and proctodaeum can thus be localized as shown in Fig. 10.4.5.

The knowledge available indicates that the mesoderm of the Diplura and Collembola has a diffuse origin over the entire area of the presumptive ectoderm, as cells which move inwards to form an inner layer beneath the ectoderm. A strong contrast is observed in Thysanura. Here, the mesoderm migrates into the interior from the midline of the disc-shaped embryonic primordium and spreads out from this site to form an inner layer of polygonal cells beneath the columnar outer layer. Thus, the presumptive mesoderm of the Thysanura has a precise location as a narrow, short, midventral area of cells in the embryonic primordium, between the presumptive stomodaeum and presumptive proctodaeum.

The presumptive ectoderm of all apterygote embryos is a bilateral area of blastoderm cells extending on either side of the ventral midline. In Thysanura the presumptive ectoderm is separate from the presumptive mesoderm and is extremely short by the time the embryonic primordium has differentiated. Presumptive head lobes are present, followed by a presumptive premandibular, mandibular, and perhaps maxillary ectoderm, but then, only a short posterior growth zone. The presumptive ectoderm of the Diplura consists of a pair of presumptive stomodaeum and including the presumptive antennal ectoderm at their posterior margins, followed by presumptive premandibular, mandibular, maxillary, labial and thoracic ectoderm, the ectoderm of the first six abdominal segements and an ectodermal growth zone in front of the presumptive proctodaeum. The several descriptions of the early germ band of collembolan embryos indicate that the presumptive ectoderm of the Collembola is similar with the exception that the posterior growth zone is not present.

Gastrulation

In apterygotes, gastrulation with respect of midgut has all but been abandoned. The presumptive midgut cells of Thysanura and Collembola become internal in different ways, during cleavage, while those of Diplura are released into the interior from the early blastoderm, retaining a slight gastrulation movement in the form of a diffuse immigration. Gastrulation with respect to the mesoderm is also a diffuse immigration throughout the length of the presumptive germ band in the Diplura and Collembola, but has gained a new precision in the Thysanura, as a rapid immigration of presumptive mesoderm cells in the ventral midline of the embryonic primordium and spread of these cells to form an inner layer. The stomodaeum and proctodaeum of apterygote embryos, like those of myriapods, penetrate the interior by independent invaginations.

Throughout the apterygotes, the stomodaeum and the proctodaeum display the simple mode of direct development already seen in myriapods. The stomodaeum always invaginates earlier in development than the proctodaeum. Once invaginated, the stomodaeum and proctodaeum (Fig. 10.4.4) continue to grow into the interior of the embryo as blind-ending tubes, the walls of which differentiate as the epithelium of the foregut or midgut respectively. The stomogastric ganglia arise as outgrowths of the dorsal walls of the stomodaeum. The Malphigian tubules develop as evaginations of the wall of the distal end of the proctodaeum. The apterygote midgut (Fig. 10.4.4.) has a simpler mode of further development. As development proceeds, many of the vitellophages within the yolk mass migrate towards the surface of the yolk and gather beneath the splanchnic mesoderm. In Collembola, the further development of the midgut epithelium proceeds in the embryo. In the Thysanura, this process is delayed until after hatching but the sequence of events is similar in both. The central part of the yolk mass tends to breakdown but the peripheral yolk subdivides into cuboidal blocks, each containing one or more peripheral vitellophages and a few vitellophages placed more deeply within the block. The peripheral vitellophages proliferate, forming small nests of cells beneath the splanchnic mesoderm. The cell nests spread out, beginning to do so earlier in the vicinity of the stomodaeum and proctodaeum than in the middle region of the embryo, and gradually appropriate the remaining yolk into a columnar epithelium which becomes the midgut epithelium. The fat-body of the apterygotes develops from mesoderm.

Development of External Form

The external development of the Thysanura is distinct from that of other apterygotes. Continuing and furthering a difference is already apparent at the blastoderm stage. We have already seen that the thysanura egg is relatively large and ovoid and that the blastoderm undergoes differentiation into a small embryonic primordium and extensive dorsal, extra-embryonic and ectoderm before the embryonic primordium begins to release mesoderm into the interior. The embryonic primordium is circular in outline when first formed, but after the inner layer has developed, the primordium begins to elongate as a germ-band (Fig. 10.4.6) and soon develops small head lobes and a short post-antennal region, which then divides into the mandibular and maxillary segments and a posterior growth zone. As the growth zone becomes active, antennal limb buds arise at the posterior margins of the head lobes, together with mandibular and maxillary limb buds on their respective segments. At this stage, the growing germ band is curved around the postero-ventral surface of the yolk mass, but an increase in length continues and the labial limb buds develop a precocious dorsal arc into the yolk (Fig. 10.4.7). By this time, the yolk mass has become temporarily cellularized into nucleated yolk spheres, which are displaced as the germ band pushes between them. Early growth and flexure of the germ band is accompanied by another precocious event, outgrowth of the labrum, combined with invagination of the stomodaeum and forward migration of the antennae

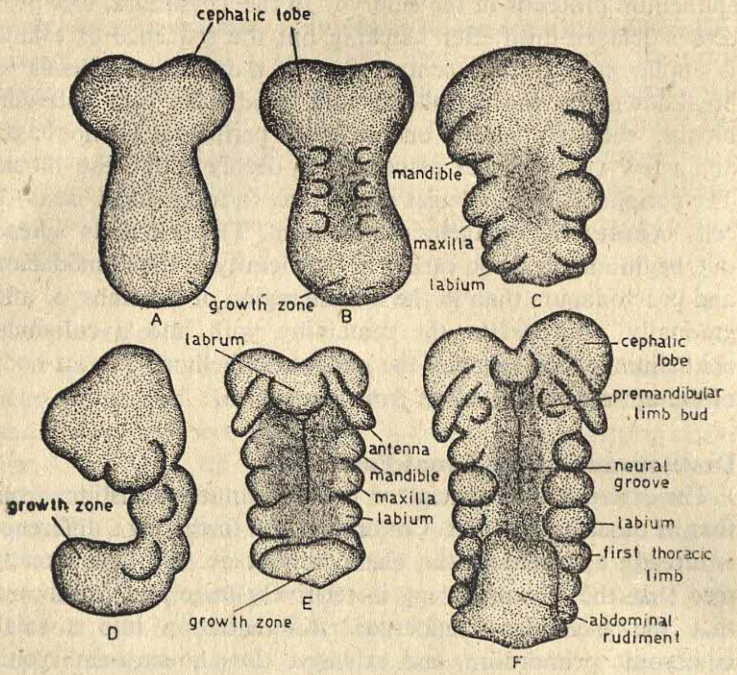


Fig. 10.4.6. Elongation and segmentation of the germ band of the thysanuran *Petrobius*.
A—Embryonic primordium.
B—Onset of segmentation.
C, D—Initial formation of limb buds.
E—Early elongation.
F—Further elongation and limb bud formation.

to their pre-oral position. As growth in length of the germ band now continues, accompanied by the serial delineation of further segments, the flexed germ band sinks more deeply into the yolk mass. Simultaneously, the margin of the germ band proliferates a sheet of small cells between itself and the margin of the superficial, extra-embryonic ectoderm. This sheet of cells forms the wall of cavity which arises as the flexed germ band sinks into the yolk mass. The extra-embryonic ectoderm of the Thysanura is now customarily called the serosa. The newly-formed cavity, roofed by the flexed germ band is the amniotic cavity and the newly proliferated wall of the cavity constitutes the amnion. It

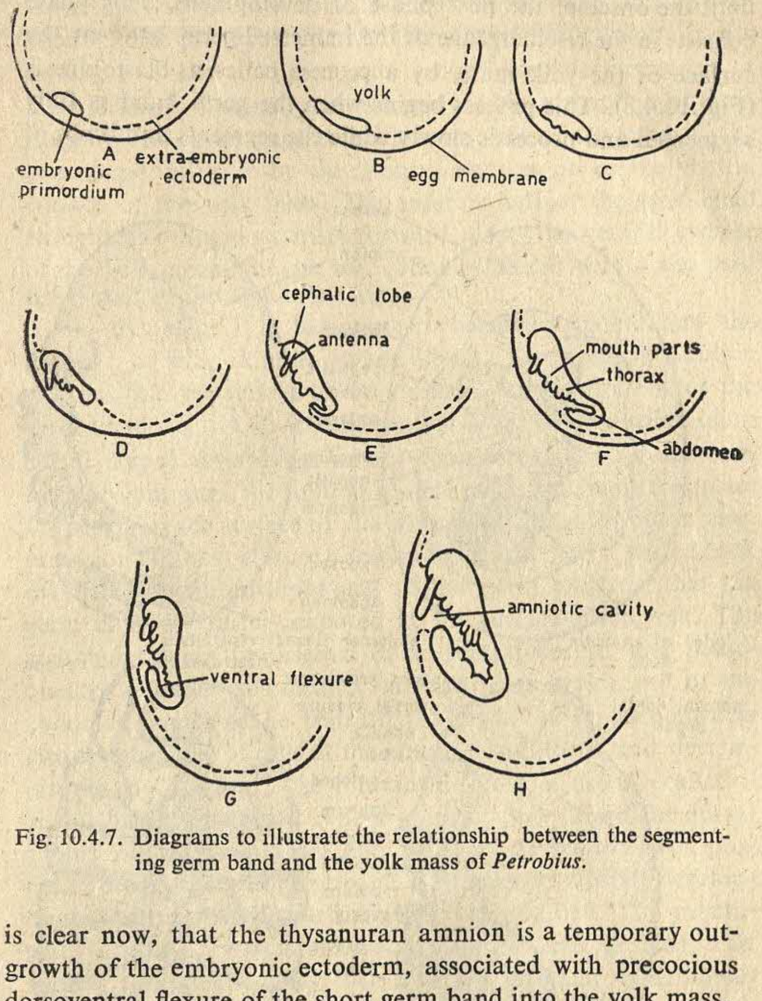


Fig. 10.4.7. Diagrams to illustrate the relationship between the segmenting germ band and the yolk mass of *Petrobius*.

is clear now, that the thysanuran amnion is a temporary outgrowth of the embryonic ectoderm, associated with precocious dorsoventral flexure of the short germ band into the yolk mass.

Once immersed in the yolk mass, the thysanuran germ band continues to increase in length through the activity of its posterior growth zone. The labial, three thoracic and ten abdominal segments are delineated in succession, leaving an eleventh abdominal unit at the posterior end of the body bearing the anus. Thoracic limb buds develop, the antennae grow longer and a pair of transient pre-mandibular limb buds develop in the region between the mandibular segment and the mouth. The development of limb buds on the abdominal segments, however, is delayed until the onset of the next phase of development. This phase consists in the re-emergence of the immersed germ band at the surface of the yolk mass, by a process called as blastokinesis (Fig. 10.4.8). This process begins when the germ band is fully segmented and proceeds slowly while the segments and limbs of

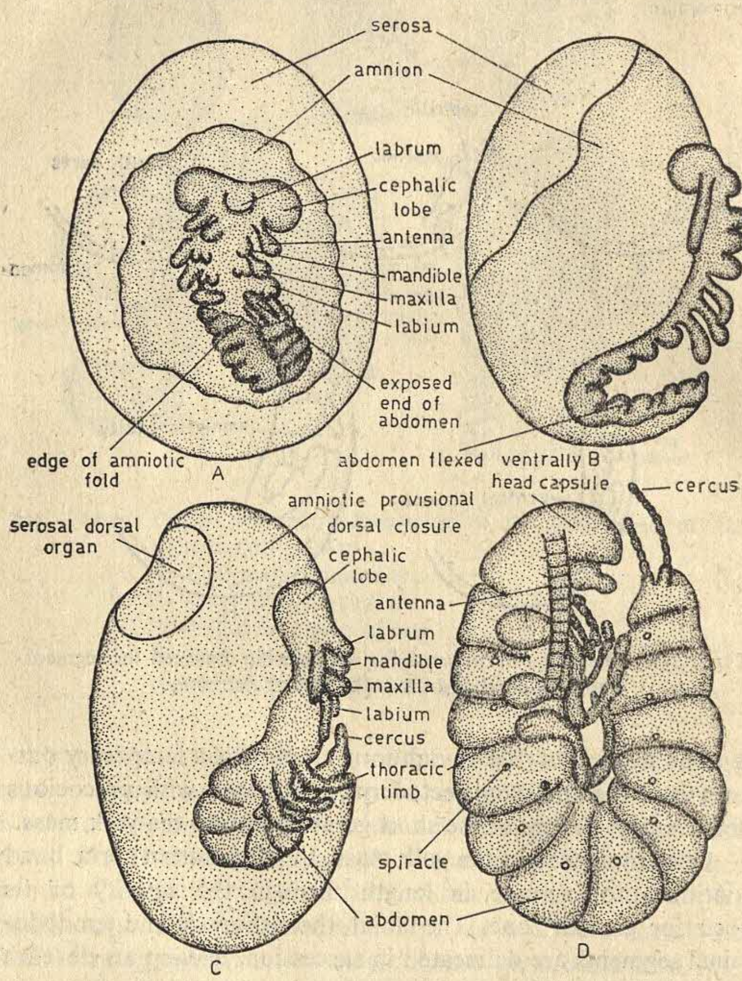


Fig. 10.4.8. Blastokinesis and dorsal closure in *Lipisma*.
A—Onset of blastokinesis.
B—Late blastokinesis.
C—Blastokinesis completed.
D—Embryo after completion of dorsal closure.

the germ band undergo further development. The first sign of blastokinesis is reopening and widening of the amniotic cavity and an eversion of the amnion around the anterior margin of this entrance. Gradually, as eversion of the amnion continues, the amniotic cavity gaps at its anterior end and the head of the germ band, followed by the thorax, emerges on to the ventral surface of the yolk mass. The anterior half of the germ band straightens out and stretches forward along the ventral surface of the yolk mass, though the abdomen flexed within the posterior part of the amniotic cavity.

By this stage it is beginning to become apparent how the process of blastokinesis comes about. The everting amnion emerges into the surface of the yolk and the anterior end of the germ band follows, in consequence of a progressive contraction of the serosa towards an antero-dorsal focus. As serosal contraction continues, the amnion stretches more and more to replace the serosa at the surface of the yolk mass, establishing a new, provisional dorsal closure. Gradually, the amnion is peeled back over the flexed posterior end of the germ band, so that the germ band is entirely exposed at the surface of the yolk. The abdomen retains the original dorso-ventral flexure, and subsequently increases this flexure, so that the posterior end of the abdomen comes to lie beneath the head. The serosa contracts into a tight knot of cells at the antero-dorsal focus and degenerates in this position. The contracted serosa is usually called a dorsal organ, though it differs in many ways from the dorsal organs of the Collembola and Diplura. As blastokinesis proceeds, the thysanuran germ band continues its further development. The pre-mandibular limb buds are resorbed. The mouthparts crowd forwards behind the mouth and develop their various component lobes. The hypopharynx and superlinguae arise midventrally between the maxillae and mandibles respectively. The antennae and thoracic limb buds increase in length. Limb buds also develop on the first and eleventh abdominal segments, then on the intervening segments.

The first pair of abdominal limb bud swells to form a pair of rounded protuberances, the pleuropodia. The pleuropodia are glandular structures and it seems likely that they secrete an enzyme which digests the serosal cuticle. Shortly before hatching takes place, the pleuropodia invaginate into the body and are resorbed. The eleventh pair of abdominal limbs persist and develop into cerci. The caudal filament is developed as a median protuberance of the posterior termination of the body, behind the anus. After the completion of blastokinesis and the tightening of the abdominal flexure, the head, thoracic segments and the abdominal segments begin to increase in length and grow dorsally around the yolk mass. Dorsal closure progresses forward from behind. The amnion is gradually replaced at the surface of the yolk by the definitive body wall and is eventually incorporated into the dorsal part of the body wall. When dorsal closure is completed, the flexed, tubular embryo occupies the entire space within the egg membranes.

It was already described how the dipluran embryo of *Campodea* develops a uniform blastoderm about an anucleate yolk mass, which is then reinvaded by cells from the blastoderm, acting as vitellophages. Differential cell division and aggregation now ensure in the ventral third of the spherical blastoderm, resulting in the formation of a hemispherical germ band in this region and an attenuated layer of dorsal extra-embryonic ectoderm over the remainder of the surface of the yolk mass. During proliferation and aggregation of the cells of the germ band, many of the cells slip beneath the surface to establish an inner layer, the mesoderm. The germ band now enters into antero-posterior elongation and becomes narrower at the same time (Fig. 10.4.2.). The anterior and posterior ends of the germ band extend on to the dorsal surface of the yolk mass and the extra-embryonic ectoderm is redistributed down the sides of the yolk mass. The anterior region of the germ band is relatively wide forming incipient head lobes. The middle region, occupying the ventral surface of the yolk mass is somewhat narrower, with a slight widening towards the posterior end.

Segmentation arises simultaneously in all but the posterior third of the germ band. At the anterior end, the head lobes broaden, the labrum and stomodaeal invagination develop in the mid-line between the head lobes and the antennal limb rudiments pouch out at the posterior margins of the head lobes, behind the stomodaeum. The first part of the post-antennal region of the germ band remains limbless at this stage, but later develops a pair of transient pre-mandibular limb buds, indicating that it is the pre-mandibular segment of the germ band.

The remainder of the anterior third becomes demarcated as the mandibular, maxillary and labial segments while the middle third becomes demarcated as the three thoracic segments and the first adbominal segment. All of these segments develop limb buds.

By the time this stage of development is reached, the posterior third of the germ band has begun to grow longer. It seems likely that the presumptive rudiment of the second to fifth abdominal segment are already present, followed by the segment-forming growth zone. As elongation continues, the end of the abdomen approaches the anterior margin of the head on the dorsal surface of the yolk mass, though the two ends always remain separated by the dorsal organ. The second to the ninth abdominal segments are delineated in succession, leaving a terminal region which carries the anus and cerci. Since the second to ninth abdominal segments develop limb buds in sequence with those of the first abdominal segment, and the cercal rudiments are the next pair of limb buds in the same sequence, it is possible that the dipluran cerci are the limbs of the tenth abdominal segment and that the terminal abdominal region on which they lie, constitutes this segment together with a terminal telson. The eighth and ninth pairs of abdominal limb buds are transient, but the pairs on segments one to seven persist and develop as styles.

Once segmentation of the germ band and formation of the limb buds is completed, a deep dorso-ventral flexure begins to develop at the mid-ventral point of the germ band, between the third thoracic and first abdominal segments (Fig. 10.4.9). As the flexure deepens, the germ band gradually folds inwards about this point, bringing the ventral surface of the thorax and head to face the ventral surface of the abdomen, and eventually bringing the anterior end of the head and the posterior end of the abdomen together at the midventral point of the egg space. At the same time, the antenna become pre-oral and the mouth parts crowd forward behind the mouth. The hypopharynx arises from the ventral maxillary ectoderm and the superlinguae from ventral mandibular ectoderm. The final posture within the egg space is similar to that observed in myriapods. As a result of dorso-ventral flexure of the germ band, the spherical yolk mass is stretched out into an elongate form and bent into a convex

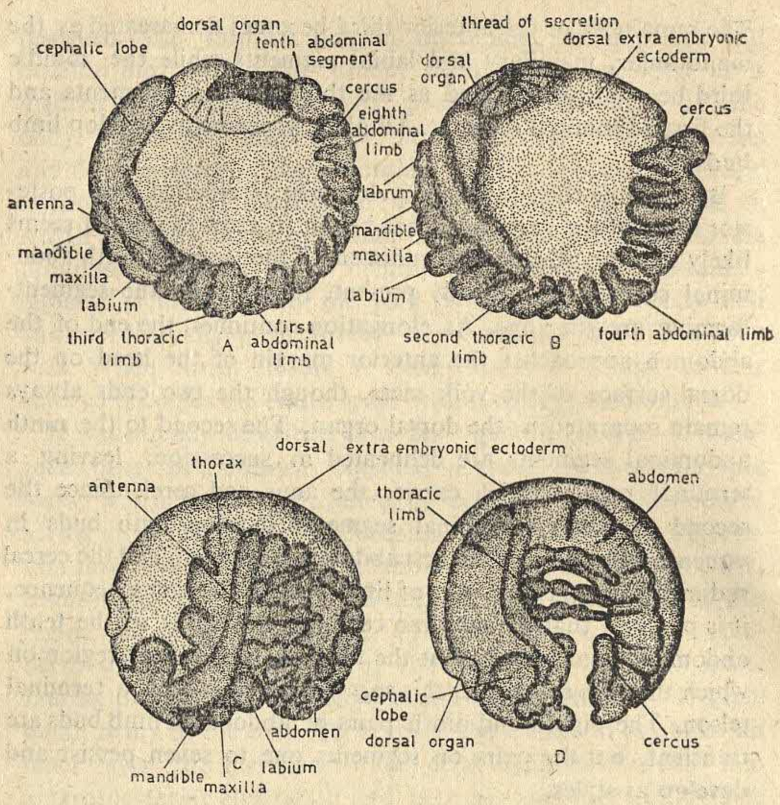


Fig. 10.4.9. Later development of *Campodea*.
A—Formation of limb buds and dorsal organ.
B—Shortening of the segmented germ band.
C—Ventral flexure.
D—Dorsal closure.

hoop above the germ band. The dorsal extra-embryonic-ectoderm is simultaneously returned to the convex dorsal surface of the yolk. The antennae, thoracic limbs and cerci increase considerably in length as the embryo undergoes dorso-ventral flexure and are accommodated with the space created by the flexure itself. Finally, upgrowth of the lateral walls of the segments effects the dorsal closure in the flexed position. Hatching takes place directly, without the intervention of a pupal phase.

The further development of the external form of the Collembola (Fig. 10.4.10) always follow the same course, whether it is

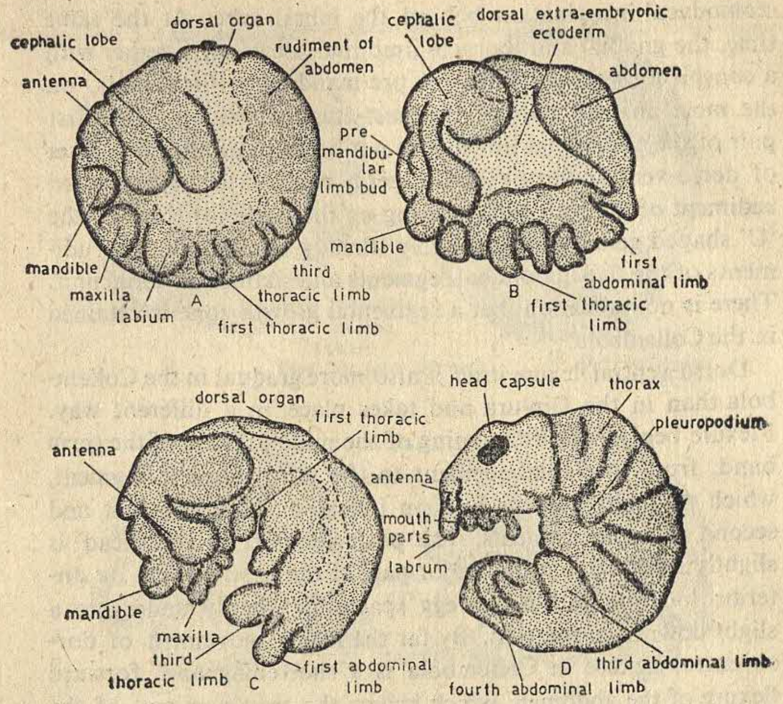


Fig. 10.4.10. Later embryonic development of the collembolan *Orchesella*.
A—Segmenting germ band.
B—Early ventral flexure.
C—Later ventral flexure.
D—Dorsal closure.

a large embryo, moderate size or small embryo. From the beginning, the embryonic primordium takes in a greater proportion of the blastoderm. When differential cell division makes the germ band externally distinct from the dorsal, extra-embryonic ectoderm, the latter can be seen confirmed to the dorsal surface of the yolk mass. The germ band changes shape and become U-shaped and the interior and posterior ends of the germ band are already close to one another on the dorsal surface before external segmentation becomes apparent.

In the first phase of external segmentation, the head lobes broaden and develops antennal buds at their posteromedial corners, a labral lobe between their anterior margins and a stomodaeal invagination behind the labral lobe. At the same time, the gnathal and thoracic limb buds develop, together with a conspicuous pair of transient pre-mandibular limb buds, on the most anterior part of the post-antennal region. The first pair of the abdominal limbs does not begin until after the onset of dorso-ventral flexure of the germ band. The unsegmented rudiment of the abdomen, making up the posterior third of the 'U' shaped germ band, probably already consists of the rudiments of the five abdominal segments and sixth or terminal unit. There is no indication that a segmental growth zone is retained in the Collembola.

Dorso-ventral flexure itself is also more gradual in the Collembola than in the Diplura and takes place in a different way. Flexure begins with a flattening of the middle region of the germ band, from the labial segment to the third thoracic segment, which gradually deepens into an inflexion about the first and second thoracic segments. The posterior part of the head is slightly inflected, but the major part of the head retains its anterior location within the egg space and simply undergoes a slight downward rotation. By far the major movement of dorsoventral flexure in Collembola is a shortening and forward flexure of the abdomen, which brings the posterior end of the abdomen forwards beneath the head. As this happens, the abdomen become segmented into five segments and a terminal unit which bears the anus. A pair of limb buds develop on the first abdominal segment at an early stage of dorso-ventral flexure. As development proceeds these limb buds come together to form the ventral tubes. Limb buds are also formed on the second and third abdominal segments. The second pair is transitory but the third pair usually develops into the hamula. A large pair of limb buds develop into the characteristic collembolan furcula or spring. The sixth or terminal unit of the abdomen, which carries the anus, may be the telson or may incorporate a sixth abdominal segment.

During dorso-ventral flexure, the yolk mass is extended and curved within the germ band in the usual way. Dorsal closure however, proceeds simultaneously with flexure and is not delayed until after dorso-ventral flexure has been completed. The development of the head takes place in the usual hexapod manner (Fig. 10.4.11). The antennae migrate forward to a pre-

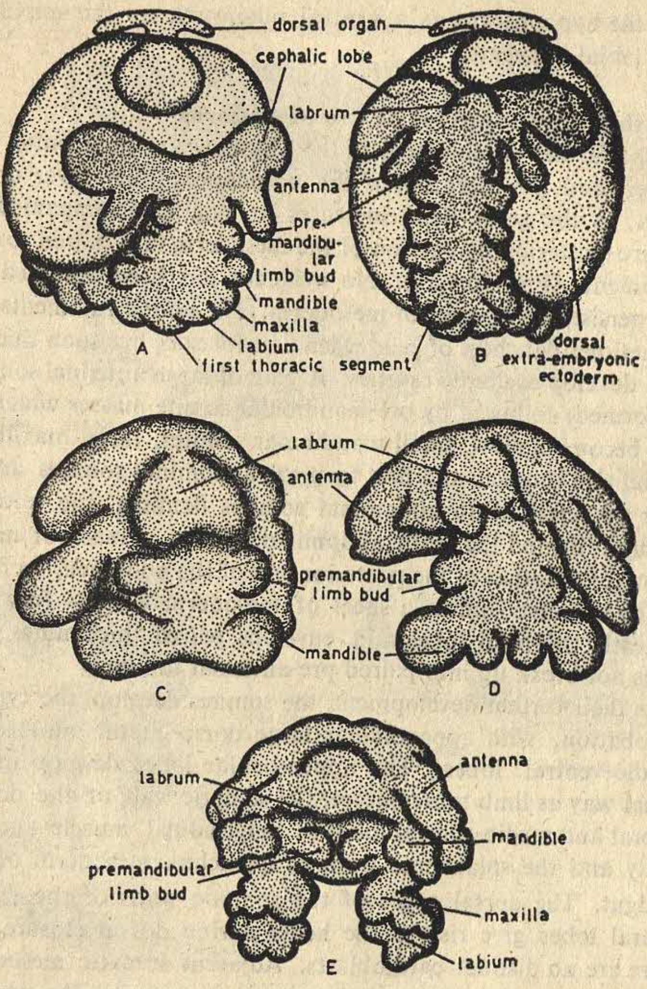


Fig. 10.4.11. Development of the head of collembola.
A, B—Anteroventral views of the anterior end of the segmenting germ band of *Anurida* showing early development of the head.
C-E—Stages in the later development of the head of *Orchesella* in ventral view.

anal position, the pre-mandibular region forms folds at the sides of the labrum and the mouth parts crowd forward to form the sides and posterior wall of the pre-anal cavity. The superlinguae arise as ventral outgrowth on the mandibular segments and the hypopharynx as a ventral outgrowth on the maxillary and labial segments.

Further Development of the Mesoderm

The further development of the Thysanura is now quite well understood. Proliferation of the mesodermal inner layer continues as the germ band increases in length, accompanied by antero-posterior segregation of the mesoderm into paired somite rudiments (Fig. 10.4.12). No evidence has been obtained for the persistence of median mesoderm. The paired rudiments are sometimes flat sheet of cells when first formed, but soon thicken and develop coelomic cavities. A pair of large antennal somites is formed, followed by pre-mandibular somite masses which do not become hollow, small mandibular somites, large maxillary, labial and thoracic and first to ninth abdominal somites and a pair of small, tenth abdominal somites flanking the proctodaeum. Behind the tenth abdominal somite, a mass of mesoderm cells persists at the posterior end of the abdomen. In front of the antennal somites a sheet of mesoderm extends forwards beneath the head lobes and envelops paired thickenings, but does not break up into paired pre-antennal somites.

In their further development, the somites develop the typical trilobation, with appendicular large dorso-lateral and small medio-ventral lobes. The appendicular lobes develop in the usual way as limb musculature, the somatic wall of the dorsolateral and medio-ventral lobes as longitudinal muscle and fat body and the splanchnic walls as splanchnic mesoderm of the midgut. The dorsal edges of the somatic walls of the dorsolateral lobes give rise to the heart during dorsal closure, but there are no distinct cardioblasts. Adjacent somatic mesoderm develops as the pericardial septum. Anteriorly, the median edges of the antennal somites come together above the stomodaeum to form the anterior aorta. The small masses of premandibular mesoderm develop into the glandular suboesophageal body. The stomodaeal and labral mesoderm arise from the sheet of mesoderm in front of the antennal somites, while the proctodaeal mesoderm is formed by the posterior mesoderm which remains behind the tenth abdominal somites.

The origin of primordial germ cells in the Thysanura is not clear. But these cells can be identified at the segmented germ

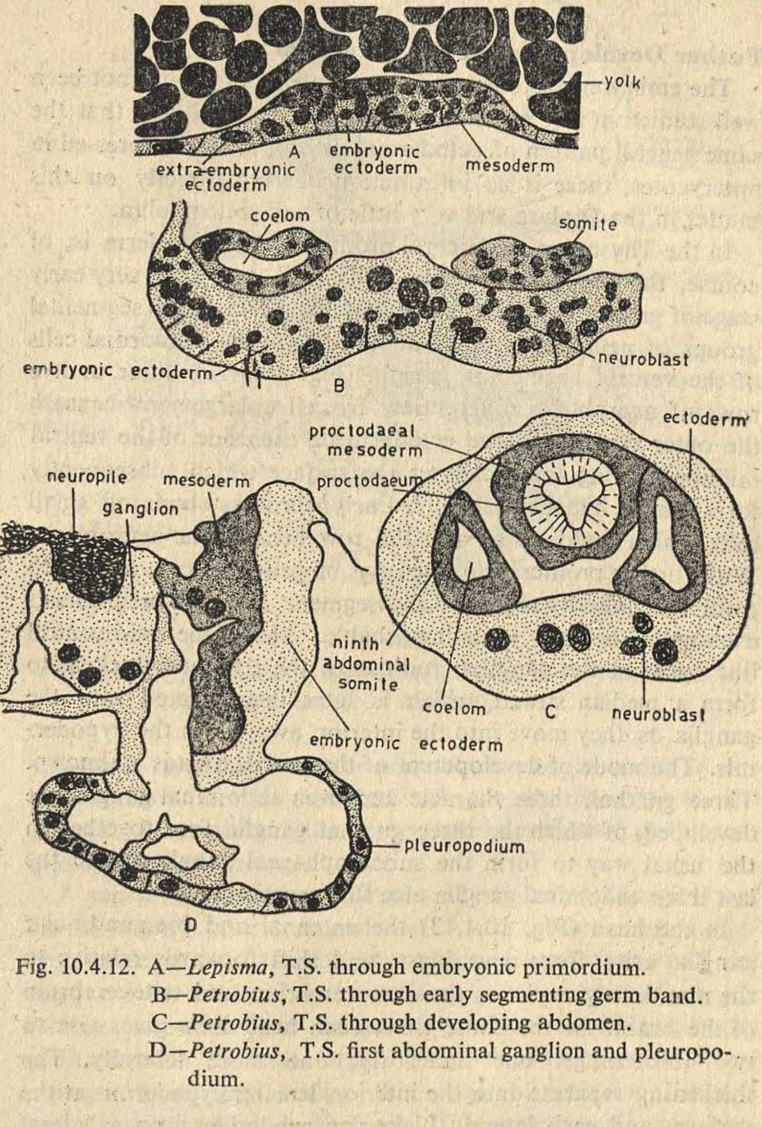


Fig. 10.4.12. A—*Lepisma*, T.S. through embryonic primordium.
B—*Petrobius*, T.S. through early segmenting germ band.
C—*Petrobius*, T.S. through developing abdomen.
D—*Petrobius*, T.S. first abdominal ganglion and pleuropodium.

band stage, as large, pale-staining cells in the splanchnic walls of the dorso-lateral lobes of several trunk somites.

The futher development of the mesoderm of the Diplura has not yet been described. With the exception of the development of the gonads, the further development of mesoderm of collembolan embryos is rather poorly known.

Futher Development of the Ectoderm

The embryonic ectoderm of apterygote embryos has not been well studied in any species. Although it seems likely that the same general pattern of ectodermal development is expressed in apterygotes, there is no information bearing directly on this matter in the Diplura and very little of any collembolan.

In the Thysanura the general product of the ectoderm is, of course, the hypodermis of the body wall, but at a very early stage of growth and segmentation of the germ band segmental groups of neuroblasts differentiate to form the primordial cells of the ventral segmental ganglia (Fig. 10.4.12). Four to five rows of neuroblasts differentiate by cell enlargement beneath the outer surfaces of the ectoderm on each side of the ventral midline, leaving small cells at the surface which subsequently form ventral hypodermal cells. Each neuroblast buds off small cells which build up as a radial row beneath the neuroblasts. With further proliferation, the rows of ganglion cells merge to form the paired ganglia of the segment and the neuroblasts eventually become indistinguishable. Along the mid-ventral line between the ganglion rudiments, the ectoderm thickens to form a median strand, which is later incorporated into the ganglia as they move into the interior, away from the hypodermis. The mode of development of the neurilemma is unknown. Three gnathal, three thoracic and even abdominal ganglia are developed, of which the three gnathal ganglia fuse together in the usual way to form the suboesophageal ganglion, and the last three abdominal ganglia also fuse together.

In the head (Fig. 10.4.13) the antennal and premandibular ganglia arise from neuroblasts and shift forwards relative to the mouth to give rise to the deutocerebrum and tritocerebrum of the brain. At the same time, each head lobe gives rise to two thickenings, one anteriorly, one more laterally. The thickening separate into the interior, leaving hypodermis at the surface, and each lateral thickening subdivides into a lateral and a posterior ganglion. Together with the anterior thickenings, which form a pair of frontal ganglia, the lateral and posterior ganglia make up the protocerebral lobes of the brain. Neither

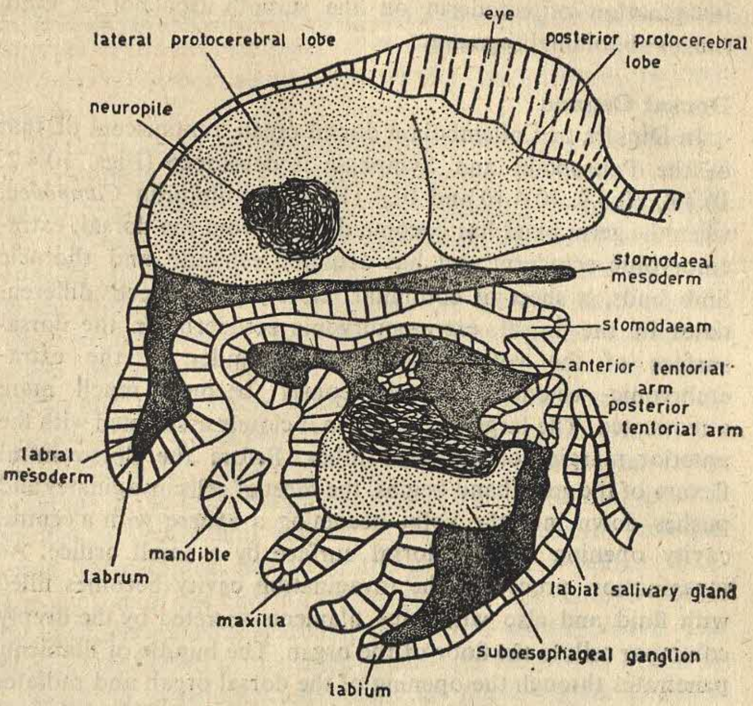


Fig. 10.4.13. Diagrammatic sagittal section through the fully developed head of the embryo of *Petrobius*.

superficial invaginations nor neuroblasts are involved in the development of the protocerebral ganglia. External to the posterior lobes of the latter, the ectoderm at the surface thickens and develop as the eyes.

A pair of salivary glands develop as ectodermal invaginations on the labial segment. In addition a pair of ectodermal invaginations develop at the anterior margin of the labium and form the posterior tentorial arms, while a similar pair of invaginations in front of the median edges of the mandibles give rise to the anterior tentorial arms. The two pairs of arms remain separate from one another. A number of ectodermal invaginations also develop on the trunk. The mesothoracic, metathoracic and first eight abdominal segments, each develop a pair of lateral invaginations which give rise to the tracheal system. An opisthogoneate gonopore is formed as a short mid-ventral invagination of ectoderm on the seventh (female) or ninth (male) abdominal segment.

Dorsal Organs

In Diplura or Collembola a dorsal organ, reminiscent of that of the Pauropoda and Symphala is developed (Figs. 10.4.2, 10.4.4, 10.4.9, 10.4.10 and 10.4.11). In the dipluran *Campodea*, when the germ band has become distinct from the dorsal, extra-embryonic ectoderm and has acquired cephalic and thoracic limb buds, a sheet of columnar, closely packed cells differentiates in the dorsal, extra-embryonic ectoderm on the dorsal surface of the yolk mass. The remainder of the extra-embryonic ectoderm simultaneously become much more attennuated. The large dorsal organ is closely associated with the anterior margins of the head lobes. Before the dorsoventral flexure of the germ band begins, the sheet of cells invaginates and pushes down into the yolk, becoming a sphere with a central cavity opening to the dorsal surface by a small orifice. As invagination proceeds, the invagination cavity becomes filled with fluid and also with blue filaments secreted by the deeply columnar cells in the floor of the organ. The bundle of filaments penetrates through the opening of the dorsal organ and radiates out between the surface of the embryo and the blastodermal cuticle. The threads are fine and delicate and do not reach the equator of the egg.

The dorsal organ of *Campodea* persists until dorso-ventral flexure of the germ band has taken place. During this process it remains attached to the margin of the head lobes and is carried over the surface of the yolk mass to an eventual antero-ventral position. Here the dorsal organ begins to degenerate, passing inwards during dorsal enclosure and eventually disintegrating within the haemocoel. The function of the dipluran dorsal organ is still unknown, but may be similar to the function of the more specialized dorsal organ of Collembola.

The dorsal organ of collembolan embryos is conspicuous in the early embryo. It arises through the enlargement of a circular patch of cells on the dorsal surface of the blastoderm, before the germ band can be distinguished. The cells elongate and the dorsal organ gradually invaginates as the germ band becomes distinct, with progressive constriction of the invagination.

During invagination, the cell boundaries disappear, the nuclei migrate to the deepest parts of the cells and the entire organ becomes mushroom shaped with fibrillar cytoplasm in the neck region.

By this time, in the small collembolan embryos, the chorion has ruptured and the blastodermal cuticle has expanded. The dorsal organ now begins to secrete radiating filaments which fan out beneath the blastodermal cuticle and soon reach the opposite pole of the egg. Fluid is secreted at the same time. When dorso-ventral flexure begins, the secreted fluid fills the space created beneath the embryo by this flexure. The dorsal organ has already begun to regress before dorso-ventral flexure begins and is gradually withdrawn into the yolk mass during the process of dorsal closure. Eventually, it degenerates within the yolk. In the unusually large embryos, the dorsal organ develops in the same manner as that of the small embryos, but reaches its maximum activity in fluid and filament secretion when dorso-ventral flexure of the germ band is taking place. The rupture of the chorion and swelling of the embryo within the blastodermal cuticle accompanies dorso-ventral flexure. The fluid is transferred to the exterior through the dorsal organ as a result of yolk digestion within the dorsal organ. It seems clear that the dorsal organ plays a part in the change of shape essential to flexure and elongation of the developing embryo and it can also be suggested that the radiating threads act in strengthening the blastodermal cuticle, which stretches to accommodate the change of shape. In small collembolan eggs, the swelling and the dorsal organ action are precocious. In the Diplura, swelling does not occur and the dorsal organ secretes only vestigeal threads, though its action in fluid secretion is still important. In the Thysanura, with a different mode of development, this type of dorsal organ is absent.

Hatching

All apterygote embryos hatch directly from their enclosing membranes without the intervention of a pupoid phase, within an embryonic cuticle such as occurs in many myriapods. The Collembola must rupture only the blastodermal cuticle in order to escape to a free life, but the Diplura still have to dispose of the chorion as well as the blastodermal cuticle at the end of development. In the Thysanura, the chorion again persists throughout development, but it seems likely that the blastodermal cuticle is digested by enzymes secreted by the pleuropodia during the later part of embryonic development, leaving only the chorion to be ruptured by the escaping first instar. The duration of development is highly variable in Pterygotes.

The Basic Pattern of Development in Apterygotes

Superficially, Diplura and Collembola appear to be remarkably myriapod like in their mode of development, while the Thysanura share many of the specializations of pterygote embryos. Each class of apterygote presents a variation on a fundamentally similar germ band. The germ band is a single, unitary structure, lacking any vestige of ventral, extra-embryonic ectoderm. The head lobes are sharply rounded in outline, project slightly in a postero-lateral direction and develop antennae at their postero-median corners. The narrow anterior end of the post-antennal region behind the head lobes is the pre-mandibular segment, which develops transient limb buds. This is followed by mandibular, maxillary and labial segments with short, forwardly directed limb buds, three thoracic segments with longer, posteriorly directed limb buds, and a series of abdominal segments with short limb buds which never develop as locomotory limbs in the manner of the thoracic limb buds, though some of them may later be involved in specialized jumping locomotion.

Associated with this type of germ band which also occurs in pterygotes, are a number of fundamental developmental features shared amongst the apterygotes and not found in myriapods. The segmental ventral ganglia develop from neuroblasts, differentiated in the ectoderm on either side of ventral midline from the antennal to the last abdominal segment. Posterior tentorial arms arise as ectodermal invaginations, on the labial segment, and a second pair of labial ectodermal invaginations give rise to the salivary glands. The pre-mandibular mesodermal somites give rise to the suboesophageal body. The gonads develop through the segmental association of primordial germ cells with splanchnic mesoderm and the gonoducts arise as segmental outgrowths of abdominal splanchnic mesoderm which link up to form paired ducts. Finally, the midgut epithelium in apterygote embryos develops from vitellophage with the yolk mass, while the fat body cells arise from segmental somatic mesoderm.

Although the functional configuration of mouthparts differs greatly in the four classes of apterygotes, the segmental composition and mode of development of the basic components of the head are identical in the Diplura, Collembola and Thysanura, despite the remarkable variations in the other aspects of their embryology.

5. PTERYGOTA

The pterygote insects have been the focus of extensive embryological research for almost a century. The easy availability of pterygotes, their often short life cycles, their economic importance and their favourable response to laboratory culture have made them attractive to embryologists. The pterygotes also display a fascinating diversity in their embryonic development, ranging from the large yolky embryos of the more primitive orders to instances of specialized polyembryony and viviparity in orders of more recent origin. The pterygote insect orders are divided into three groups and their embryology described separately.

1) Hemimetabola (Odonata, Ephemeroptera, Dictyoptera, Isoptera, Plecoptera, Cheleutoptera, Orthoptera, Embioptera and Dermaptera).

2) Paraneoptera (Psocoptera, Mallophaga, Anoplura, Thysanoptera, Homoptera and Heteroptera).

3) Holometabola (Coleoptera, Megaloptera, Neuroptera, Simphonaptera, Hymenoptera, Mecoptera, Trichoptera, Lepidoptera and Diptera).

Development of Hemimetabola

In spite of the variations in shape, the eggs of the primitive Hemimetabola always have at least one dimension close to or greater than 1 mm. The generalized shape is ovoid, with distinct anteroposterior and dorsoventral areas, and it is not uncommon for the ventral surface to be convex and the dorsal surface concave (Fig. 10.5.1). The egg contains very little cytoplasm, the bulk of its substance being made up of closely packed yolk spheres. The nucleus is usually centrally placed in a small cytoplasmic halo. A thin periplasm has been identified in certain odonatan, orthopteran and dermapteran eggs but is said to be absent in other species of these orders.

Even though the eggs of pterygotes vary markedly in size and structural configuration, their basic and almost universal mode of cleavage is the same intralecithal mode described for Thysanura (Figs. 10.5.1 and 10.5.2). Nuclear mitoses always synchronous at first and often synchronous throughout cleavage, proceed without corresponding division of the cytoplasm. Cytoplasmic

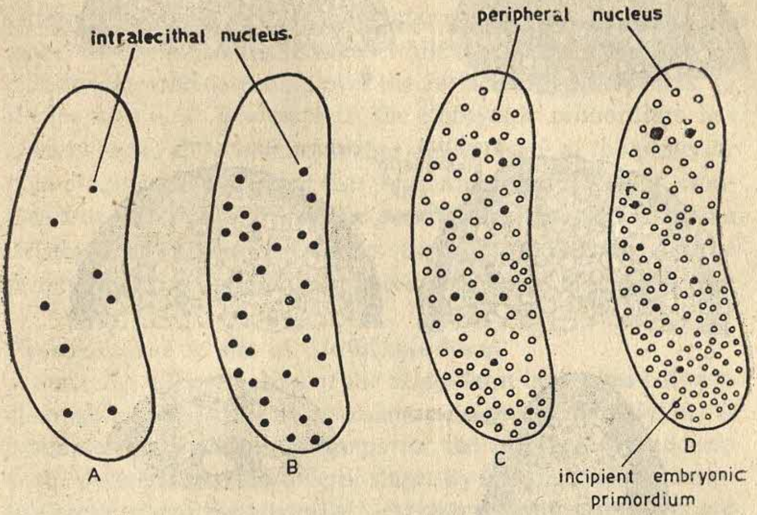


Fig. 10.5.1. Stages in the cleavage of *Kalotermes* (Isoptera).
A—8-nucleus stage.
B—32-nucleus stage.
C—140-nucleus stage.
D—194-nucleus stage.

haloes accumulate around the daughter nuclei, forming cleavage energids. The energids spread through the yolk mass and gradually approach the periphery. In most pterygote eggs, some of the cleavage energids remain within the yolk mass as vitellophages. In many primitive Hemimetabola the cleavage energids arrive uniformly at the surface of the yolk mass. The next event is the formation of a blastoderm. Many species follow the same pattern of blastoderm formation of the Thysanura. In the primitive hemimetabolan orders blastoderm formation results from the division and spread of the peripheral cleavage energids, which are separated from one another when they first emerge. An attenuated, uniform blastoderm results from this process. Blastodermal differentiation then ensures, resulting in the formation of an embryonic primordium as a compact disc or band of columnar cells, ventrally or posteroventrally placed on the surface of the yolk mass. A more precocious differentiation of the embryonic primordium during blastoderm formation is seen in the Dictyoptera, Isoptera, Cheleutoptera and many

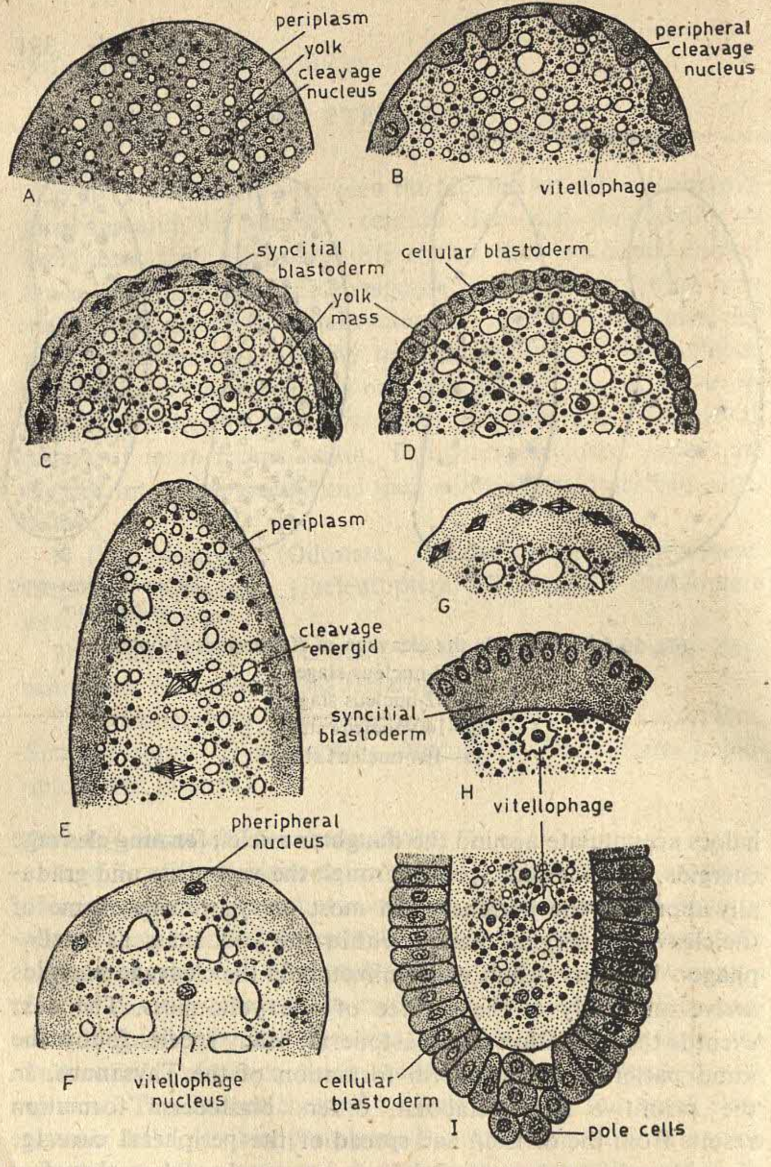


Fig. 10.5.2. Stages in blastoderm formation of *Bruchidius* (Coleoptera) and *Dacus* (Diptera).

A—2-nucleus stage.
B—256-nucleus stage.
C—Approximately 4000-nucleus stage.
D—Blastoderm.

Bruchidius

E—16-nucleus stage.
F—512-nucleus stage.
G—Approximately 4000-nucleus stage.
H—Approximately 8000-nucleus stage.
I—Posterior end of cellular blastoderm and pole cells.

Dacus

Orthoptera. In these orders, cellular concentration to form the embryonic primordium proceeds while the cleavage energids are still dividing and spreading over the surface of the yolk mass. In the Isoptera and Cheleutoptera, the embryonic primordium has already begun to enter into further development at the posterior pole of the egg by the time that blastoderm formation is completed at the anterior pole. In general, the completion of the differentiated blastoderm marks the end of cleavage in the primitive Hemimetabola and precedes the onset of gastrulation.

Presumptive Areas of the Blastoderm

Since the differentiation of the blastoderm intervenes between cleavage and the onset of gastrulation in pterygote embryos, it is simplest to construct fate maps for the pterygote blastoderm at the differentiated blastoderm stage. By this time, all presumptive areas except the attenuated, extra-embryonic ecotoderm are concentrated within the embryonic primordium. The blastodermal fate map is remarkably stable throughout the pterygotes. In most pterygote embryos, the cells of the embryonic rudiments are difficult to distinguish from one another until gastrulation is well advanced, so that the interpretation of their prior arrangement in the blastoderm is a difficult problem. The relative positions of the rudiments when they become distinct and the manner in which the blastoderm cells arrive at these positions make it possible to postulate where the cells lay prior to gastrulation. The presumptive areas of the blastodermal differentiation can therefore be summarized as follows. The embryonic primordium comprises:

Presumptive mesoderm, a narrow midventral band of cells;

Presumptive anterior midgut and presumptive posterior midgut, small areas of cells at the anterior and posterior ends respectively of the band of presumptive mesoderm;

Presumptive stomodaeum, an arc of cell around the anterior margin of the presumptive anterior midgut;

Presumptive proctodaeum, a small area of cells behind the presumptive posterior midgut; and

Presumptive embryonic ectoderm, paired ventrolateral bands of cells which border the presumptive stomodaeum, anterior midgut, mesoderm, posterior midgut and proctodaeum, and meet in the anterior midline in front of the presumptive stomodaeum.

The anterior parts of the presumptive ectoderm in front of and around the presumptive stomodaeum, anterior midgut and anterior part of the mesoderm, take the form of broad head lobes. These are made up of the presumptive ectoderm of the pre-antennal part of the head, together with antennal ectoderm at the posteromedian corners of the head lobes. The remainder of each ectoderm band is narrower. The narrow part of the band comprises a sequence of presumptive pre-mandibular, gnathal and thoracic segmental zones followed by a terminal growth zone from which the abdominal ectoderm arises. In primitive hemimetabolous orders, all of the presumptive ectoderm behind the antennal level is concentrated into a growth zone from which the postantennal ectoderm subsequently arises by proliferation. The functional significance of this secondary specialization of development is not clear.

Around the grouped presumptive areas of the embryonic primordium, the remainder of the surface of the yolk mass is occupied by presumptive extra-embryonic ectoderm which is broadly attenuated. The yolk mass contains vitellophages additional to the presence of the anterior and posterior midgut rudiments in the embryonic primordium.

GASTRULATION

In accordance with the strict localization of the presumptive mesoderm along the ventral midline of the embryonic primordium the gastrulation of pterygote embryos centres around the entry of midventral cells into the interior, accompanied by the formation of a temporary midventral groove, the gastral groove (Fig. 10.5.3). This grove may be long or short, deep or shallow, transient or relatively persistent, depending on the species, but is always closed before the external delineation of the segments of the embryo begins. The edges of the paired bands of presumptive ectoderm then come together in the ventral midline, closing the gastral groove. The mesoderm spreads out as a layer of cells beneath the embryonic ectoderm. In discussing gastrulation it is not easy to separate the movements of the mesoderm from those of the presumptive midgut rudiments. In most species, the midgut cells, which lie at the two ends of the

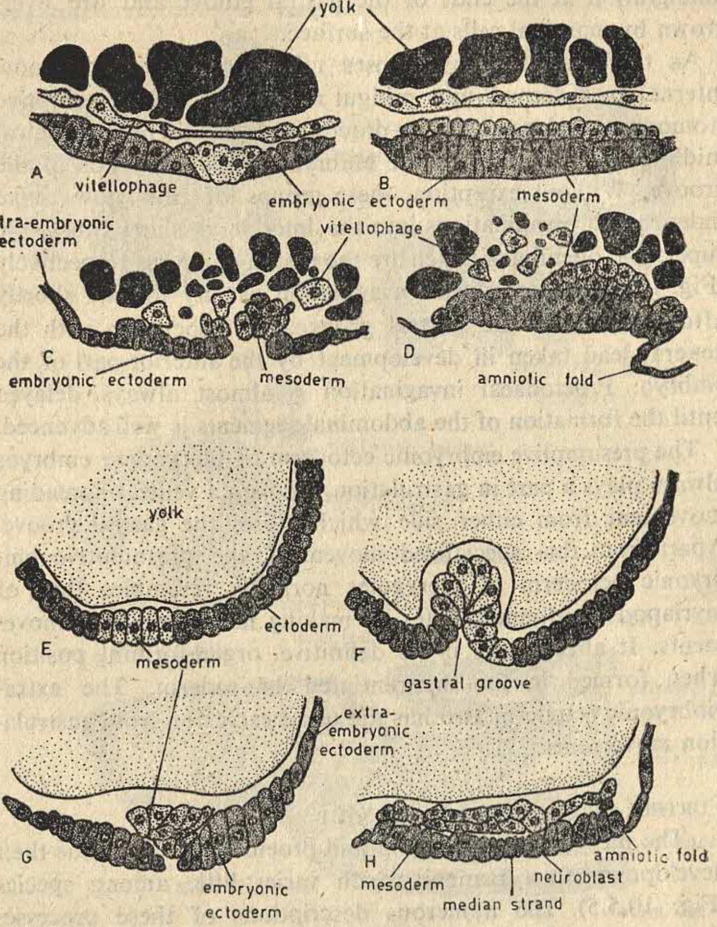


Fig. 10.5.3. A—T.S. through embryonic primordium of *Carausius*.
B—T.S. through early germ band of *Carausius*.
C—Sagittal section through the embryonic primordium of *Kalotermes* during gastrulation.
D—Sagittal section through the early germ band of *Kalotermes*.
E to H—Stages in gastrulation of *Chrysopa*.

mesoderm, cannot usually be distinguished from the mesoderm histologically until gastrulation is complete. The presumptive anterior and posterior midgut cells move into the interior by immigration at the ends of the gastral groove and are overgrown by marginal cells at the surface.

As the gastral groove closes midventrally over the now internal mesodermal and midgut rudiments, the presumptive stomodaeal and presumptive proctodaeal cells lie in the ventral midline at the surface of the embryo, at the two ends of the groove. Without exception, these groups of cells now make independent invaginations into the interior as short tubes, the superficial opening of which are the mouth and anus respectively (Fig. 10.5.4). Stomodaeal invagination usually follows shortly after closure of the gastral groove, in association with the general lead taken in development by the anterior part of the embryo. Proctodaeal invagination is almost always delayed until the formation of the abdominal segments is well advanced.

The presumptive embryonic ectoderm of pteryogote embryos always plays a part in gastrulation, through a ventral spreading movement from either side which closes the gastral groove. Apart from this specialized movement, the presumptive embryonic ectoderm of pterygotes normally resembles that of myriapods and apterygotes in making no gastrulation movements. It already lies in its definitive, organ-forming position when formed in the differentiated blastoderm. The extra-embryonic ectoderm also has the usual exclusion from gastrulation movements.

FURTHER DEVELOPMENT OF THE GUT

The pterygote stomodaeum and proctodaeum continue their development in a manner which varies little among species (Fig. 10.5.5). The numerous descriptions of these processes have almost always been associated with accounts of development of the midgut and can therefore be cited in connection with the latter topic. The stomodaeum grows inwards and backwards as a simple epithelial tube, reaching as far as the thorax. The distal end of the tube becomes reflexed on itself, forming the central part of the proventriculus. The remainder of the stomodaeal wall differentiates as the epithelium of the oesophagus. Three median outgrowths arise from the dorsal wall of the stomodaeum either as thickenings or evaginations and give rise to the ganglia of the stomogastric nervous system. The proctodaeum also grows inwards as an epithelial tube and

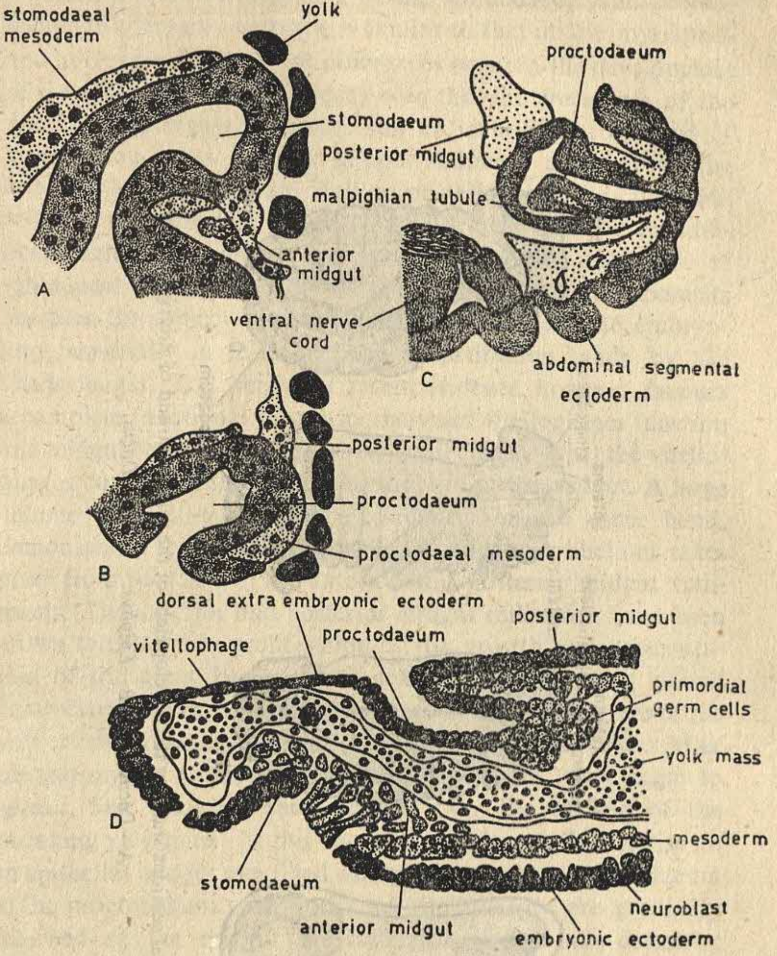


Fig. 10.5.4. A—The stomodaeum and anterior midgut rudiment of *Carausius*.
B—The proctodaeum and posterior midgut rudiment of *Carausius*.
C—The proctodaeum and posterior midgut of *Rhadnius*.
D—The anterior half of the gastrula of *Dacus*.

develops as the lining epithelium of the hindgut. The distal end of the proctodaeum becomes pouched out as the rudiments of the Malpighian tubules, which grow as blind ending tubes into the haemocoel.

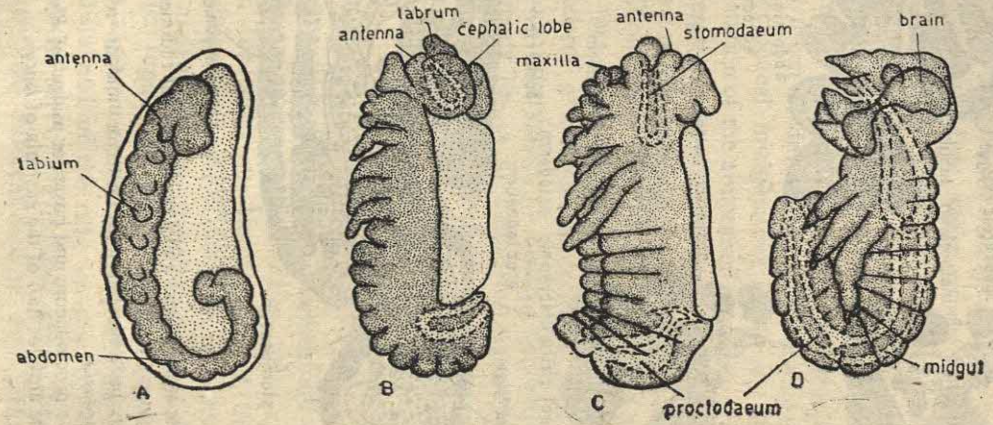


Fig. 10.5.5. Sagittal sections showing the development of the midgut strand of pterygote embryos.

A—*Locusta*. B—*Epiphys*. C-D—*Dacus*.

Although the development of the stomodaeum and proctodaeum in pterygote embryos is similar to that of the myriapods and apterygotes, important differences occur in the development of the midgut. We have already seen that the precursors of the midgut in pterygote embryos can be identified at the end of gastrulation as an anterior midgut rudiment attached to the end of the stomodaeum and a posterior midgut rudiment attached to the end of the proctodaeum (Fig. 10.5.4). In addition, vitellophages are present in the yolk mass. The mode of subsequent development of the midgut from these components has been the subject of much controversy in pterygote embryology, especially in relation to the contribution made by the vitellophages. The weight of recent evidence, however, favours a complete functional separation between vitellophages function and midgut formation. There is no good evidence that the vitellophages contribute structurally to the wall of the midgut. A large volume of well-substantiated evidence, on the other hand, demonstrates that the formation of the midgut epithelium takes place from the cells of the anterior and posterior midgut rudiments. The anterior and posterior midgut rudiments have been shown to enter into proliferation as the growth and segmentation of the germ bands nears completion, producing paired ventrolateral strands of cells which grow along the surface of the yolk mass and meet in the middle region of the embryo. After the two midgut strands have become continuous, they begin to spread, first ventrally, then dorsally over the surface of the shrinking yolk mass. In this way the midgut strands give rise to an epithelial midgut sac, filled with yolk, joining the stomodaeum to the proctodaeum. The yolk and vitellophages are gradually resorbed as the midgut sac differentiates into the definitive midgut epithelium. In spite of continuing controversy, we can now be certain that midgut development in the pterygotes proceeds in a fundamentally different manner from that of apterygotes, in spite of the many similarities between pterygote and thysanuran embryos. It should be noted that the pterygote fat body like that of apterygotes, develops from mesoderm cells.

DEVELOPMENT OF EXTERNAL FORM

Although in general the development of the external form of pterygote embryos follows the lines already described for the

Thysanura, there are manifold variation and complications in the different orders. Some are related to the functional specializations of the newly hatched young but others are temporary embryonic peculiarities. The development of all pterygote embryos is epimorphic, so that the full complement of segments is formed and functional in all species when hatching takes place. The location of the small embryonic primordium on the surface of the yolk mass varies considerably. Most frequently the embryonic primordium lies in a postero-ventral portion (Figs. 10.5.6 and 10.5.7). The shape of the primordium also varies. The head lobes are formed as paired enlargements of the anterior part of the primordium. As already described the head lobes comprises the pre-antennal and antennal regions of the embryo. The remainder of the primordium, or postantennal region is relatively long and includes the rudiments of the gnathal and thoracic segments as well as the posterior growth zone (Figs. 10.5.6, 10.5.7, 10.5.8 and 10.5.9). This form probably constitutes the basic type of pterygote embryonic primordium.

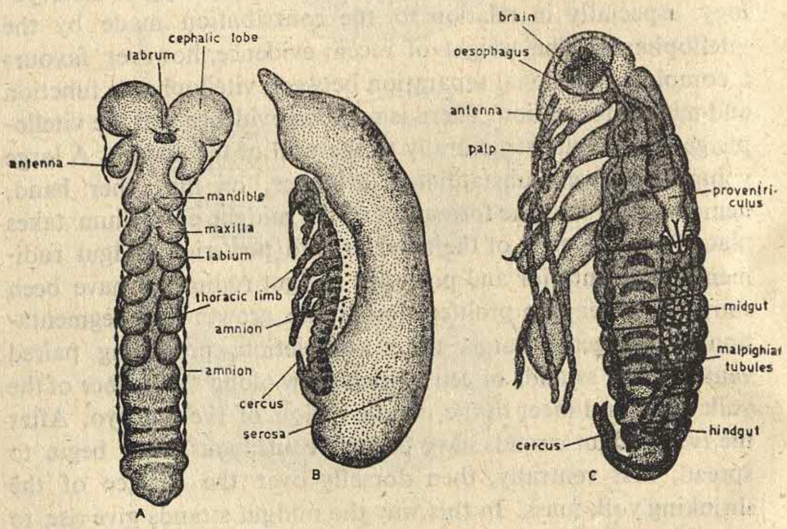


Fig. 10.5.6. The external features of development of *Blatta*.
A—Segmenting germ band, ventral view.
B—Embryo during Kretatrepsis, left lateral view.
C—Embryo approaching hatching.

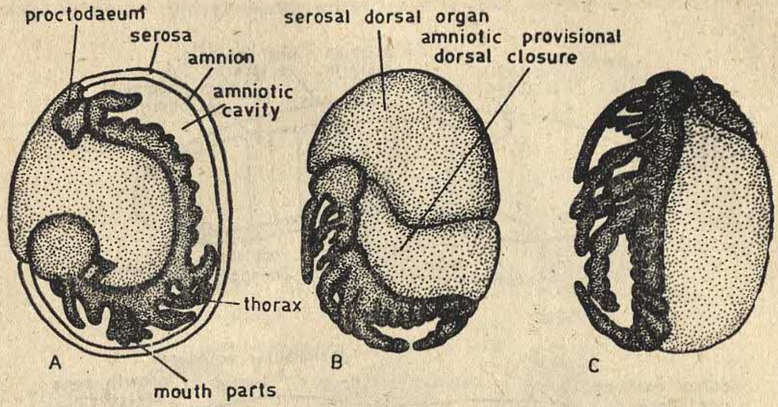


Fig. 10.5.7. The external features of development of *Forficula*.
D—Segmented germ band
E—Early katatrepis.
F—Katatrepsis completed.

Accompanying and following the onset of gastrulation, the embryonic primordium begins to increase in length and shortly after this, the external delineation of segments begins. The segments are usually delineated in anteroposterior succession, though some primitive Hemimetabola begin this process at the third thoracic segment and proceed both anteriorly and posteriorly from this point. In the simplest pattern of growth of segmenting (Figs 10.5.6 and 10.5.7) the growth proceeds in a posterior direction along the ventral surface of the yolk mass, accompanied by a slight forward shift of the anterior end of the germ band and a downward flexure of the posterior end when it reaches the posterior pole of the yolk mass.

In other primitive Hemimetabola, the dorsoventral curvature of the growing germ band becomes more pronounced, resulting in partial or complete immersion of the germ band in the yolk mass. The specialized combination of growth and movement which brings the germ band into this position is called anatrepis. The dorsoventral curvature of the pterygote germ band is in the opposite direction to the dorsoventral flexure which carries the thysanuran germ band deep into the yolk mass.

During the growth and segmentation of the germ band, limb buds develop on the head and thorax, usually in anteroposterior

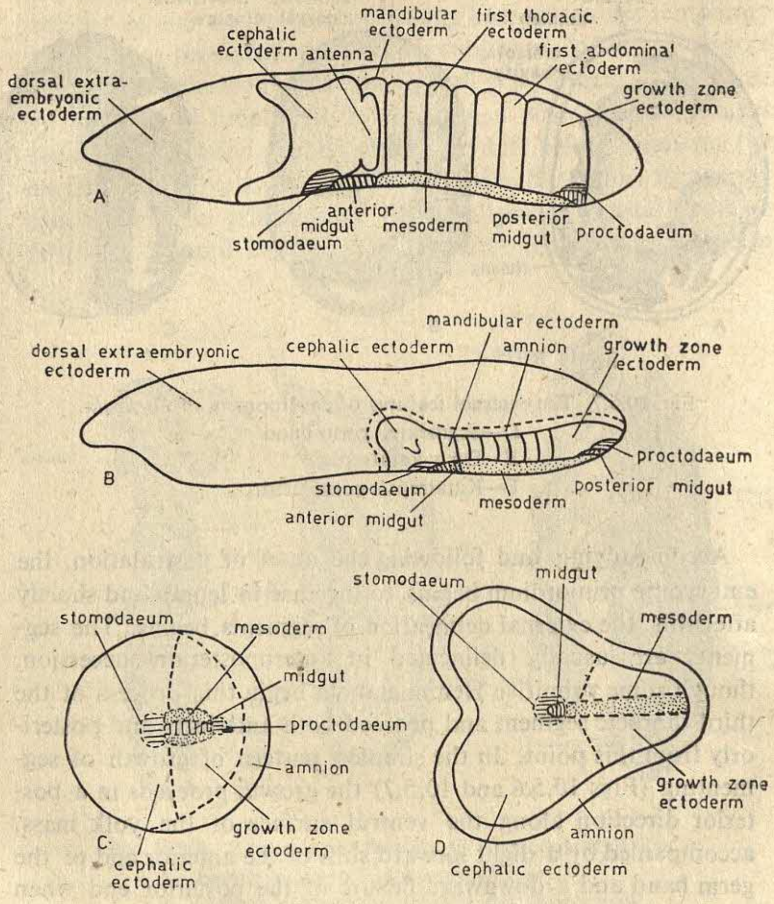


Fig. 10.5.8. Fate maps of pterygote blastoderm.

A—The blastoderm of *Platycnemis* in left lateral view at the uniform blastoderm stage.

B—The differentiated blastoderm of *Platycnemis* in left lateral view.

C—The embryonic primordium of *Kalotermes*, ventral view.

D—The embryonic primordium of *Carausius*, ventral view.

succession. The antennal limb buds arise at the posteromedian corners of the head lobes, behind the level of the invaginating stomodaeum and are followed by the gnathal and thoracic limb buds in their respective segments. The labrum pouches out

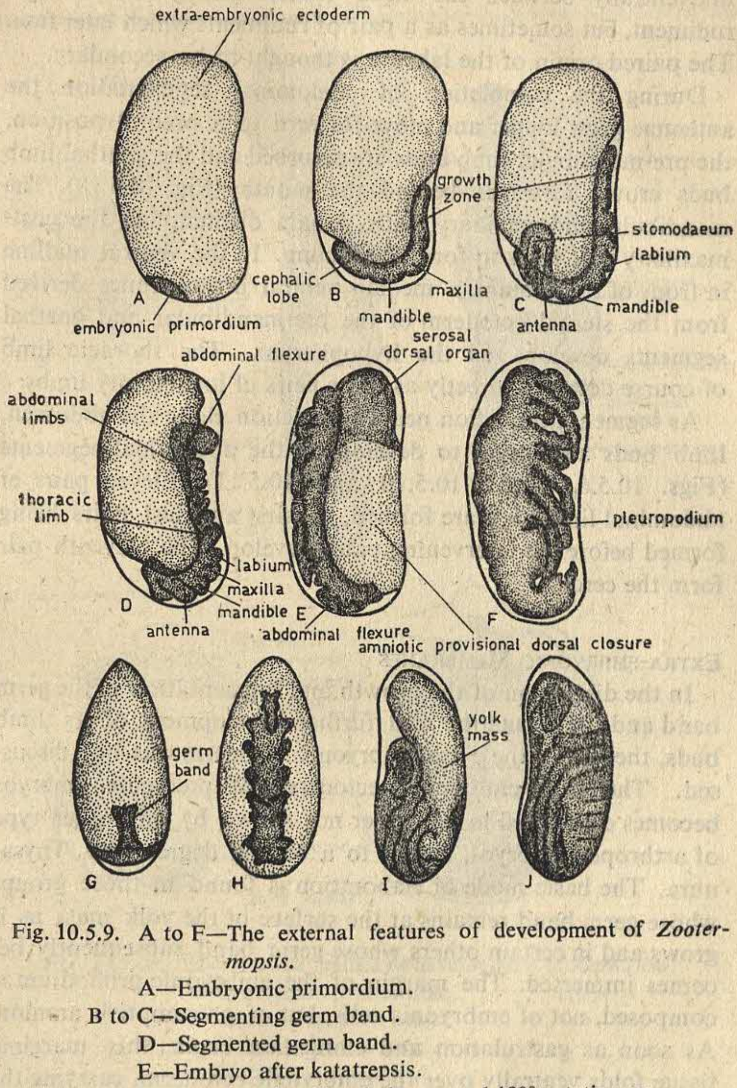


Fig. 10.5.9. A to F—The external features of development of *Zootermopsis.*

A—Embryonic primordium.

B to C—Segmenting germ band.

D—Segmented germ band.

E—Embryo after katatrepsis.

F—Embryo after dorsal closure and 180° rotation on long axis.

G to J—The external features of development of *Saldula.*

G—Early growth of germ band and anatrepis.

H—Segmented germ band immersed in yolk.

I—Embryo during katatrepis.

J—Embryo after dorsal closure.

midventrally between the head lobes, usually as a single rudiment, but sometimes as a pair of rudiments which later fuse. The paired origin of the labrum is thought to be secondary.

During the completion of abdominal segmentation the antennae grow longer and move forward to a preoral position, the pre-mandibular limb buds are resorbed and the gnathal limb buds crowd forwards behind the mouth (Fig. 10.5.10). The mandibular and maxillary pairs remain distinct, but the post-maxillary pair fuses to form the labium. In the ventral midline in front of the labium, a median ventral protuberance derived from the sternal ectoderm of the pre-mandibular and gnathal segments develops as the hypopharynx. The thoracic limb of course develops directly as three pairs of locomotory limbs.

As segment delineation nears completion along the abdomen, limb buds also begin to develop on the abdominal segments (Figs. 10.5.6, 10.5.7, 10.5.10 and 10.5.11). Eleven pairs of abdominal limb buds are formed, the first and last pairs being formed before the intervening pairs develop. The eleventh pair form the cerci.

EXTRA-EMBRYONIC MEMBRANES

In the discussion of the growth and segmentation of the germ band and the formation and further development of its limb buds, the role of the extra-embryonic ectoderm was not discussed. The extra-embryonic ectoderm of pterygote embryos becomes elaborated in a manner not shown by any other type of arthropod embryos, except to a certain degree, the Thysanura. The basic mode of elaboration is found in those groups whose germ band remains at the surface of the yolk mass as it grows and in certain others whose germ band subsequently becomes immersed. The margin of the embryonic primodium is composed, not of embryonic cells, but of presumptive amnion. As soon as gastrulation and elongation begin, this marginal tissue folds ventrally over the embryonic ectoderm, carrying the edge of the attenuated, extra-embryonic ectoderm with it (Fig. 10.5.12). As the folds meet and merge in the ventral midline, the germ band becomes covered by a double layer of extra-embryonic ectoderm. The resulting inner layer is the amnion, enclosing an amniotic cavity outside the ventral surface of the germ band. The outer layer, continuous over the yolk mass,

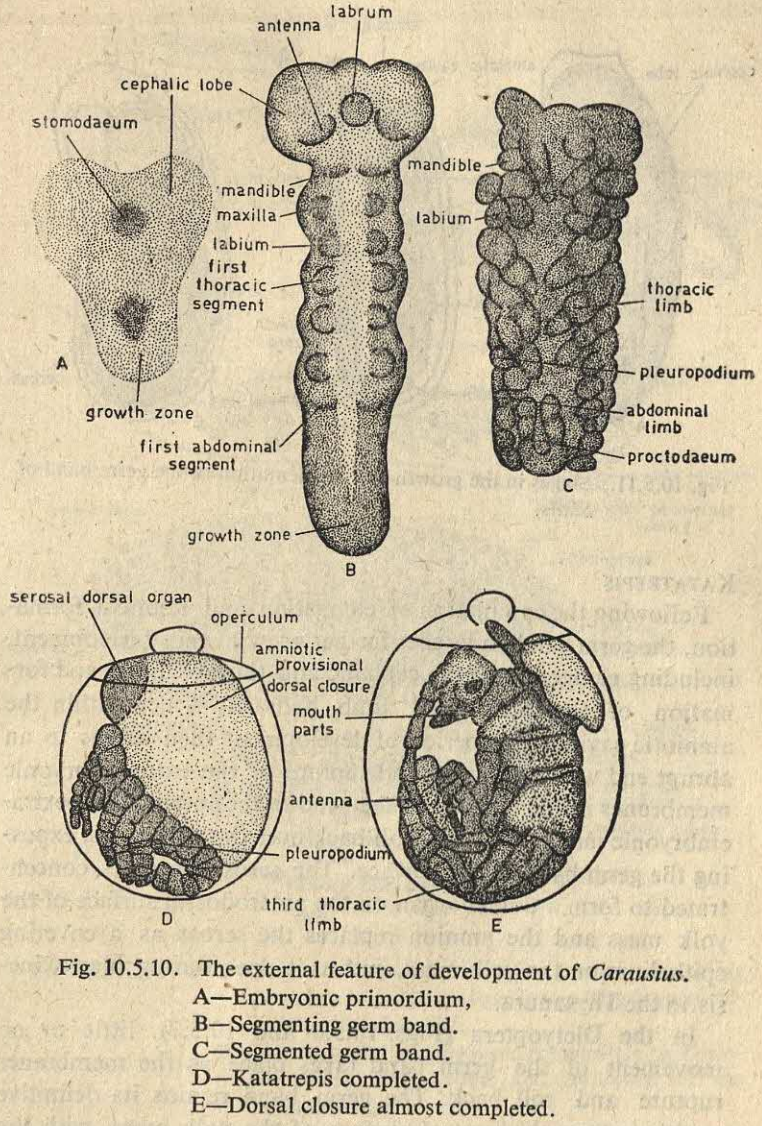


Fig. 10.5.10. The external feature of development of *Carausius*.
A—Embryonic primordium,
B—Segmenting germ band.
C—Segmented germ band.
D—Katatrepis completed.
E—Dorsal closure almost completed.

is the serosa. The serosa secretes a serosal cuticle beneath the vitelline membrane soon after amnio-serosal separation has occurred.

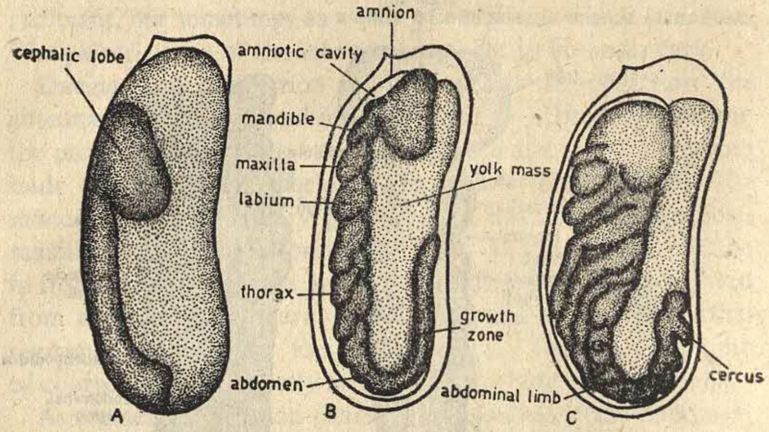


Fig. 10.5.11. Stages in the growth and segmentation of the germ band of *Sialis*.

KATATREPIS

Following the completion of elongation and segment formation, the germ band undergoes further growth and development, including elongation of the cephalic and thoracic limbs and formation of the abdominal limb buds, as it lies within the amniotic cavity. This period of development then comes to an abrupt end with the fusion and rupture of the extra-embryonic membranes in the vicinity of the head of the embryo. The extra-embryonic membranes now roll back over the yolk mass, exposing the germ band at the surface. The serosa becomes concentrated to form a dorsal organ on the anterodorsal surface of the yolk mass and the amnion replaces the serosa as a covering epithelium on the yolk mass, just as it does during blastokinesis in the Thysanura.

In the Dictyoptera (Figs. 10.5.6 and 10.5.7), little or no movement of the germ band takes place as the membranes rupture and roll back. The germ band retains its definitive position along the ventral surface of the yolk mass, with the posterior end of the abdomen flexed downwards and forwards. All other hemimetabolous embryos, however, reach this stage with the germ band on the head facing the posterior pole. Several orders have the germ band immersed in the yolk as a result of antepis (Figs. 10.5.6, 10.5.7 and 10.5.13). Rupture of

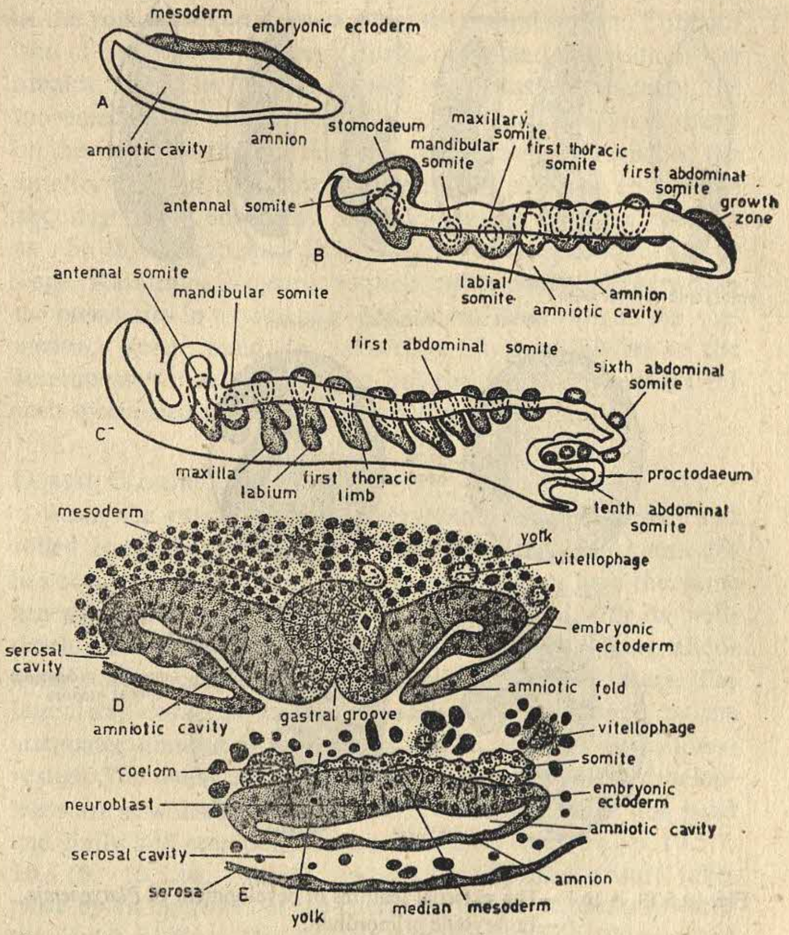


Fig. 10.5.12. A to C—Stages in somite formation in the segmenting germ band of *Kalotermes*, drawn diagrammatically in left lateral view.
D—T.S. through the embryonic primordium of *Tenebrio* during gastrulation and formation of the amniotic folds.
E—T.S. through the segmenting germ band of *Tenebrio*.

extra-embryonic membranes in these species is always followed by a rapid migratory movement which brings the germ band

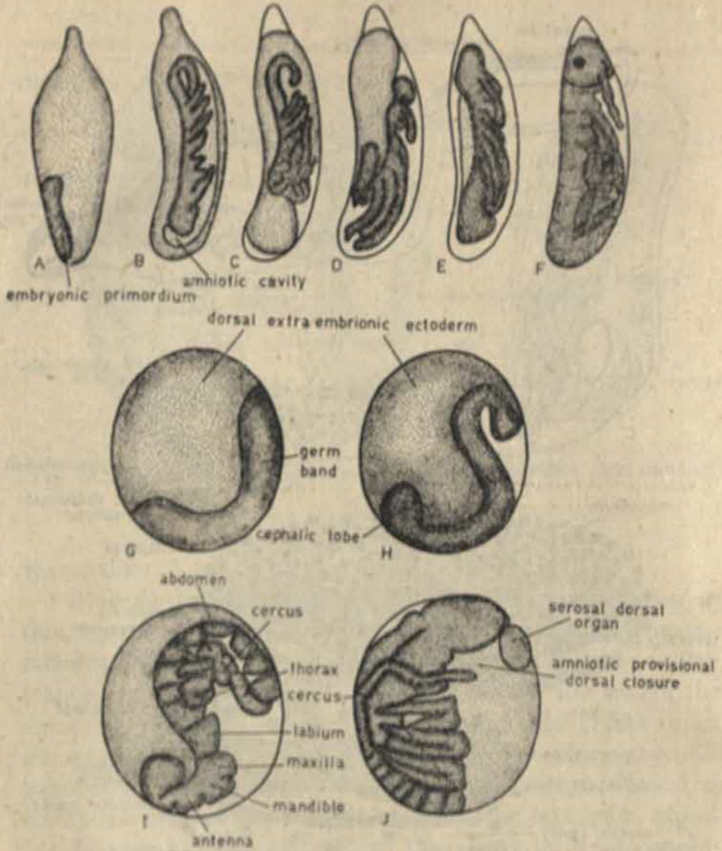


Fig. 10.5.13. A to F—The external features of development of *Platycnemis*.
A—Embryonic primordium.
B—Segmented germ band after anatropis.
C—Onset of katatrepis.
D and E—Stages in katatrepis.
F—Dorsal closure completed.
G to J—External features of development of *Baetis*.
G—Embryonic primordium.
H—Segmenting germ band.
I—Segmented germ band before katatrepis.
J—Embryo after katatrepis.

into its definitive position relative to the yolk mass. This movement is called katatrepis. Usually, katatrepis is a straight migratory movement of the germ band over the posterior pole of the yolk mass and forward along the ventral surface. Contraction of the serosa to form a dorsal organ and reflection of the amnion over the surface of the yolk mass accompany the movement. The general result is to establish the germ band on the ventral surface of the yolk, with the anterior end at the anterior pole of the yolk mass and the posterior end of the abdomen flexed downward and forward, in the same position as the thysanuran and dictyopteran germ band at the same stage. Katatrepis is clearly a specialized process evolved within the pterygotes in association with the earlier growth of the segmenting germ band in the wrong direction relative to the anteroposterior axis of the egg, but the functional reasons for such specialized procedures remain obscure.

DORSAL CLOSURE

When the extra-embryonic membranes have ruptured and rolled back, the serosal dorsal organ has formed and katatrepis has occurred, the majority of pterygote embryos have the same general configuration. The segmental germ band with its well-developed cephalic and thoracic limbs and its developing abdominal limbs, occupies the ventral surface of the yolk mass. The lateral and dorsal surfaces of the yolk mass are covered by the attenuated amnion and the serosa is reduced to an anterodorsal vestige. The major events in the completion of external development are now dorsal closure, further development of the head and limbs and secretion of the exoskeleton (Figs. 10.5.6, 10.5.7, 10.5.10, 10.5.14, 10.5.15 and 10.5.16). Dorsal closure takes place by an upgrowth of the lateral wall of the segments towards the dorsal midline. The dorsal organ is simultaneously resorbed into the yolk mass, followed by the shrinking vestige of the amnion.

Throughout the external development of the more generalized pterygote embryos, the sequence of events is highly reminiscent of the external development of the Thysanura. The only marked difference is that the pterygotes no longer retain the dorsoventral flexure of the germ band that the thysanura share in common with the other apterygotes.

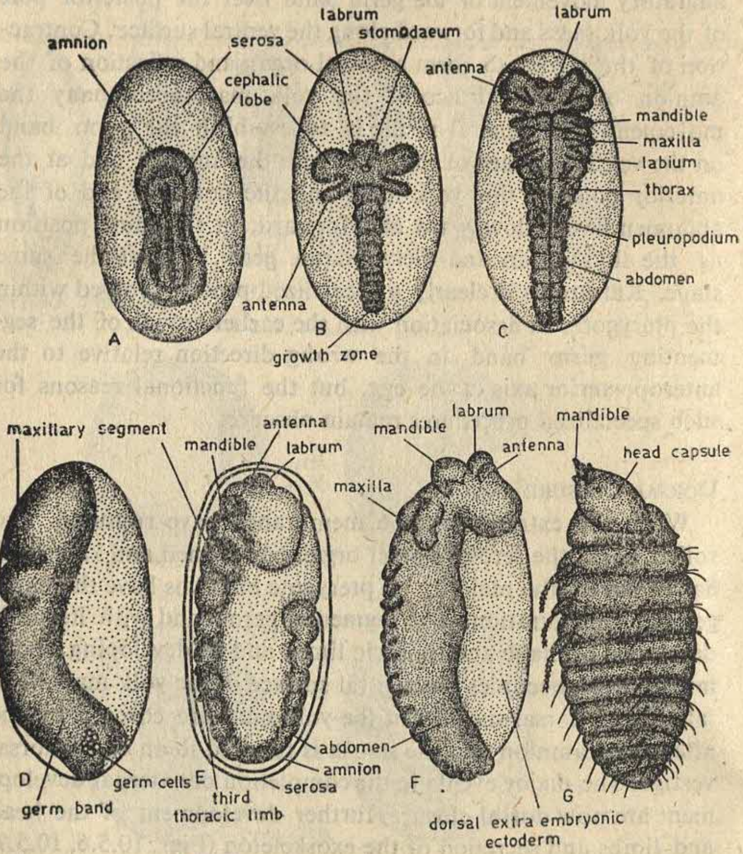


Fig. 10.5.14. A to C—Stages in the growth and segmentation of the germ band of *Tenebrio*.

D to G—The external features of development of *Bruchidius*.

D—Early segmenting germ band.

E—Segmented germ band.

F—During shortening of germ band.

G—About to hatch.

FURTHER DEVELOPMENT OF THE MESODERM

As soon as the gastrulation movement of the mesoderm into the interior is completed in a pterygote embryo and the mesoderm cells have spread out as a single layer beneath the ectoderm of the germ band, further changes begin. Anteriorly, the mesoderm cells begin to express the first stages of organogeny.

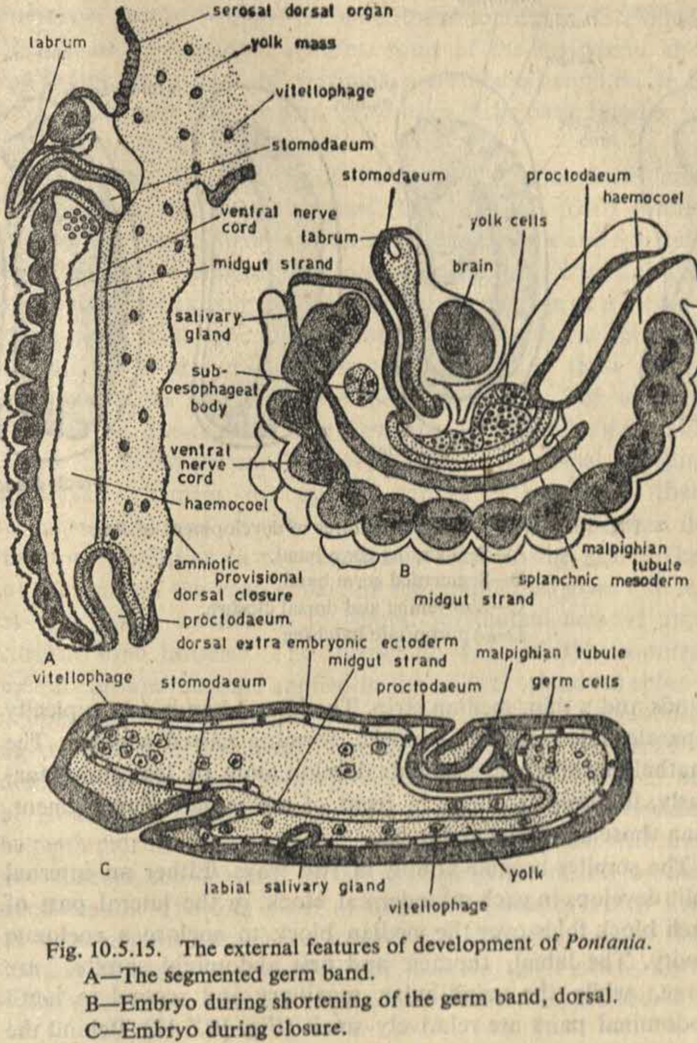


Fig. 10.5.15. The external features of development of *Pontania*.
A—The segmented germ band.
B—Embryo during shortening of the germ band, dorsal.
C—Embryo during closure.
D—Embryo approaching hatching.

Posteriorly, proliferation of new cells usually continues, giving rise to the mesoderm of further segments.

The first stage in the further development of the mesoderm (Fig. 10.5.12) is a bilateral concentration of the mesoderm cells towards the sides of the germ band, giving two, thick lateral

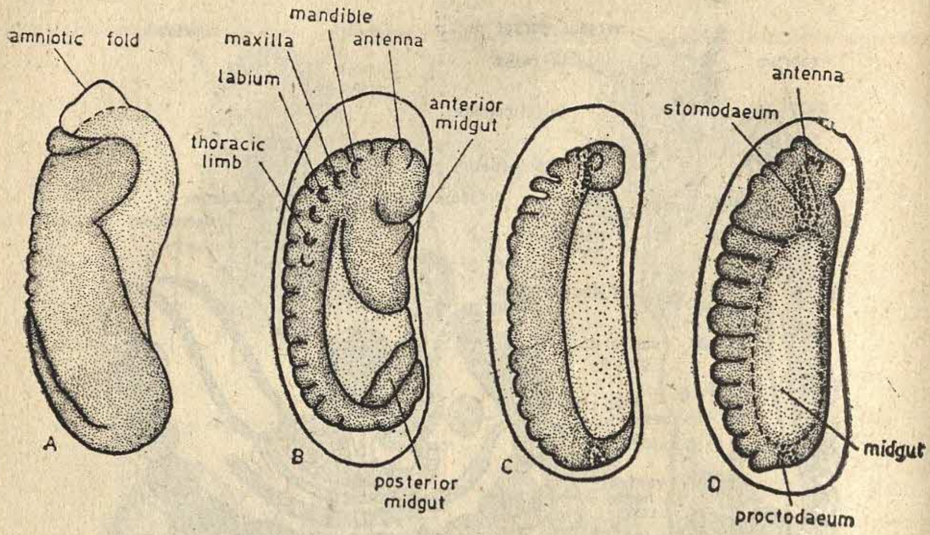


Fig. 10.5.16. The external features of development of *Apis*.
A—Segmenting germ band.
B—Segmented germ band.
C—Shortening and dorsal closure.
D—Appropriate hatching.

bands and a thin, median strip. The lateral bands now typically separate into segmental blocks, forming paired somites. The gnathal and thoracic segments develop more or less simultaneously, followed by those in front of the mandibular segment, than those along the abdomen.

The somites become hollow in two ways. Either an internal split develops in each mesodermal block, or the lateral part of each block folds over the median block to enclose a coelomic cavity. The labial, thoracic and first abdominal somites are large, while the mandibular, maxillary and second to tenth abdominal pairs are relatively small (Fig. 10.5.12). Behind the tenth pair of abdominal somites, a small mass of mesoderm persists around the proctodaeum.

In front of the mandibular somites, the mesoderm continues forward on either side of the stomodaeum, beneath the head lobes, and meets anteriorly beneath the labrum. A large antennular pair is formed just behind the level of the stomodaeum.

Then, in the mesoderm linking the antennal to the mandibular somites, a pair of vestigial premandibular somites is developed. In front of the antennal somites, some of the mesoderm also aggregates as a pair of vestigial pre-antennal somites. It is generally agreed that six pairs of somites is the basic number in the developing peterygote head.

The further development of the somites is remarkably uniform throughout the pterygotes (Fig. 10.5.17). Each somite pouches out ventrolaterally as an appendicular lobe and expands dorsally as a dorsolateral lobe, although the ventral part of the somite does not extend towards the ventral midline as a medioventral lobe. The appendicular lobe gives rise to the intrinsic musculature of the attendant limb bud, except in those abdominal segments that later lose their limb buds. The somatic wall of the dorsolateral lobe develops as dorsal longitudinal muscle, fat body, pericardial septum and, at its dorsal margin, cardioblasts. During the upward spread of mesoderm that accompanies dorsal closure, the cardioblasts come together in the dorsal midline in the usual way to form the heart. The somatic wall of the ventral part of the somite also gives rise to fat-body cells, as well as to ventral logitudinal muscles and extrinsic limb muscles. The splanchnic walls of the somites separate off and become applied to the midgut strands as splanchnic mesoderm. In addition to giving rise to the splanchnic musculature and additional fat body cells, the splanchnic mesoderm of pterygote embryos is the site of development of the gonads and genital ducts. The residual mesoderm behind the tenth pair of abdominal somites become associated with the proctodaeum and develops as the splanchnic mesoderm of the proctodaeum. The thin sheet of median mesoderm which lies between the paired segmental somites normally gives rise to the haemocytes, although the origin of haemocytes is often more diffuse in embryos in which somite formation is suppressed.

CEPHALIC SOMITES

Even though the labial somites of generalized pterygote embryos retain a pattern of development similar to that of the thoracic somites, the development of the more anterior cephlic somites displays the usual specializations. The small mandibular and maxillary somites retain only a limited developmental

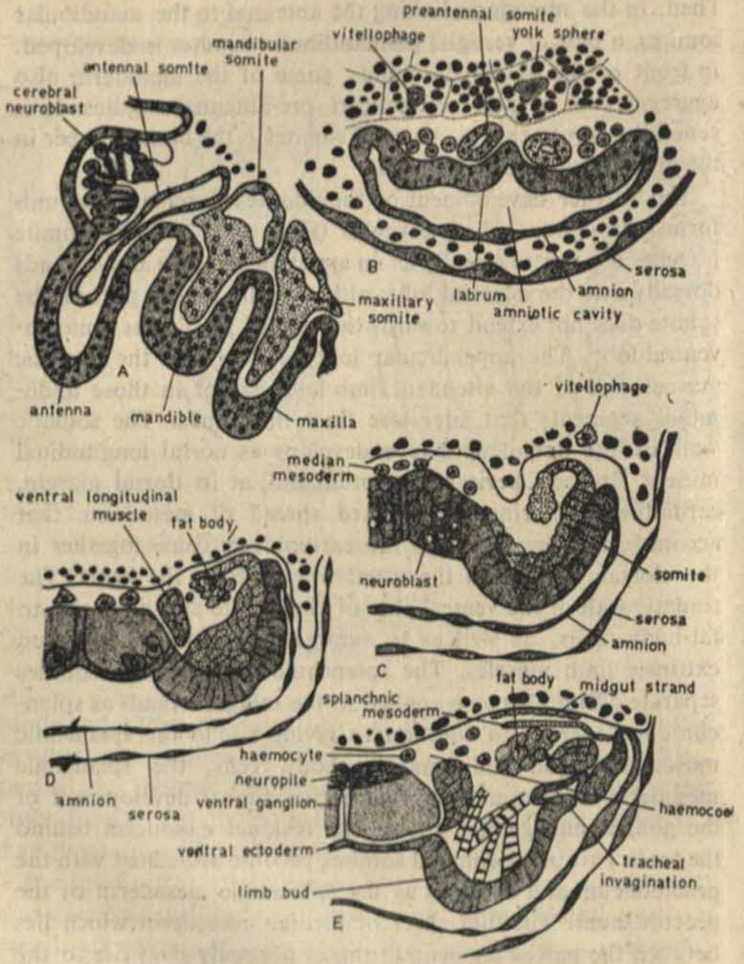


Fig. 10.5.17. A—Parasagittal section through the antennal and gnathal segments of *Tenebrio* at a stage when the gnathal limb buds are beginning to crowd forwards around the mouth.

B—T.S. through the labral region of the segmented germ band of *Tenebrio*.

C to E—Stages in the further development of a thoracic somite of *Chrysopa*.

capacity giving rise to no more than the extrinsic and intrinsic limb muscles of their respective segments. The pre-mandibular somites are even more specialized. They give rise to the glandular suboesophageal body. The large antennal somites retain more of the basic features of somite development. The appendicular lobe is typical. The somatic wall of the rest of the somite forms fat body, while the splanchnic walls of the paired somites come together above the stomodaeum to form the anterior aorta. In general, the pre-antennal somites and their associated median mesoderm develop as the labral and other musculature at the front of the head.

Gonads and gonoducts: The primordial germ cells either first become recognizable in the splanchnic walls of the certain abdominal somites or are segregated from the posterior ends of the mesoderm during gastrulation and migrate subsequently to the splanchnic walls of the abdominal somites. Once in this location (Fig. 10.5.18) the segmental group of germ cells proliferate and merge to form two continuous strands, each covered by a thin splanchnic epithelium. The gonads arise from these strands.

FURTHER DEVELOPMENT OF THE ECTODERM

Like the embryonic ectoderm of Thysonura, the pteryogote embryonic ectoderm exhibits an early differentiation of neuroblasts as a preliminary to the proliferation of segmental ganglia (Figs. 10.5.12 and 10.5.17). As the activity of the growth zone adds progressively to the posterior end of the germ band, the mandibular and succeeding ectoderm develops a longitudinal thickening on either side of the ventral midline, producing paired neural ridges bordering a neural groove. The thickening is due to the proliferative activity of three to five rows of neuroblasts formed by the enlargement of ventral ectoderm cells. Through teloblastic budding, the neuroblasts proliferate radial rows of small cells inwards as ganglion cells. The neuroblasts sink slightly into the interior as they bud, leaving other ectoderm cells as dermatoblasts at the surface. Between the neural ridges, a median strand of tall ectoderm cells forms the floor of the neural groove, with occasional neuroblasts differentiate along its length.

As the number of cells proliferated by the neuroblasts increases, the radial cell rows merge to form ganglionic cell masses associated with the paired mesodermal somites. In front of the mandibular segment, the neuroblast rows diverge on

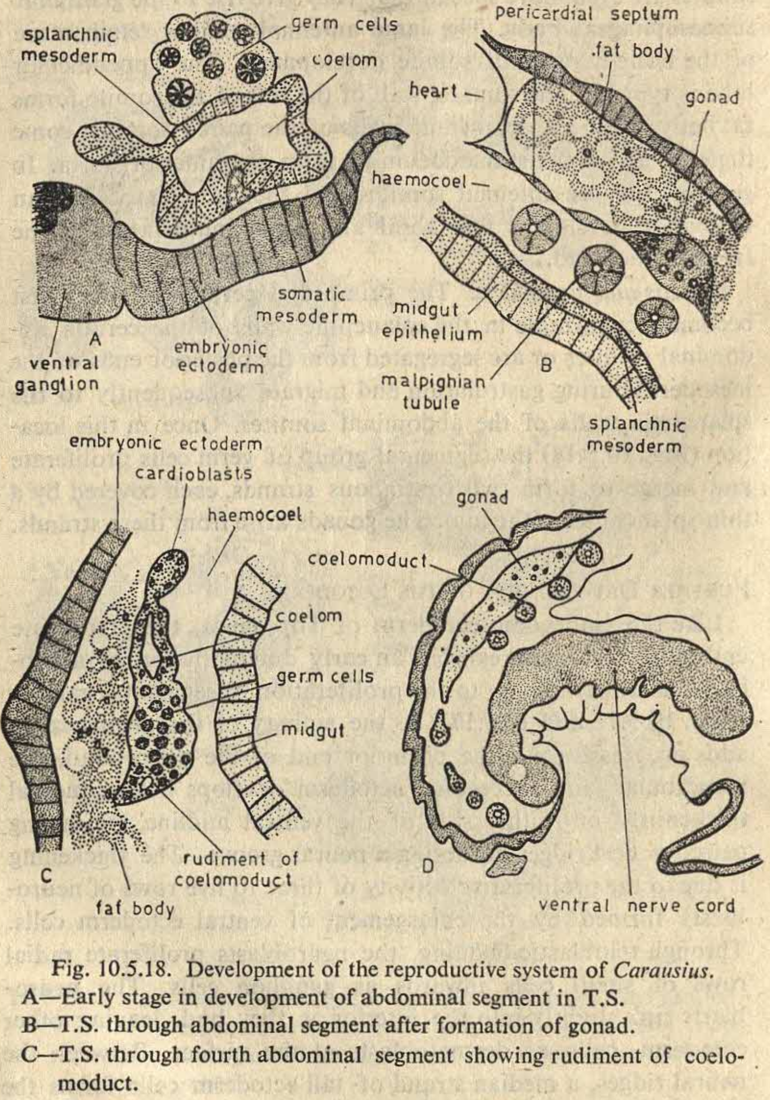


Fig. 10.5.18. Development of the reproductive system of *Carausius.*
A—Early stage in development of abdominal segment in T.S.
B—T.S. through abdominal segment after formation of gonad.
C—T.S. through fourth abdominal segment showing rudiment of coelomoduct.
D—Parasagittal section through the abdomen of an embryo showing arrangement of gonad and coelomoducts.

either side of the stomadaeum and are much broader over the anterior parts of the head lobes. The neuroblasts give rise to paired pre-mandibular or tritocerebral ganglia behind the stomodaeum, paired antennal or deutocerebral ganglia on either side of the stomodaeum, and large, paired protocerebral masses in front of the stomodaeum (Fig. 10.5.19). The early develop-

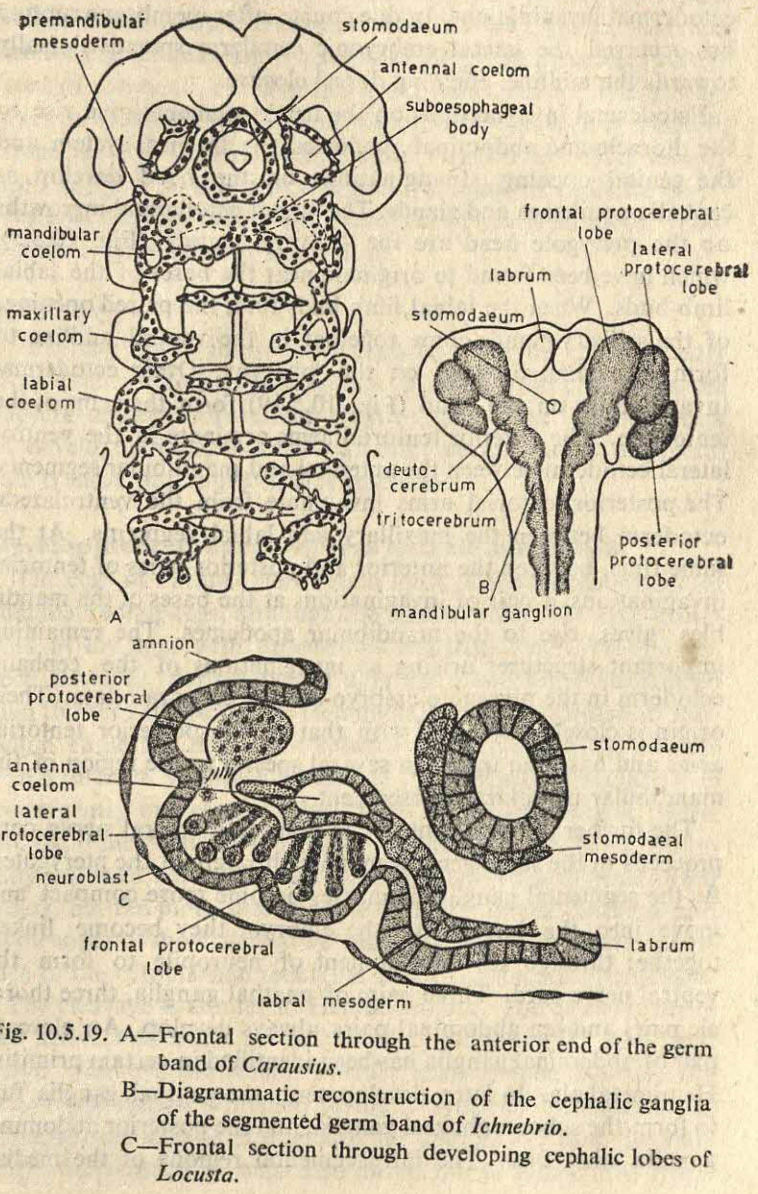


Fig. 10.5.19. A—Frontal section through the anterior end of the germ band of *Carausius*.
B—Diagrammatic reconstruction of the cephalic ganglia of the segmented germ band of *Ichnebrio*.
C—Frontal section through developing cephalic lobes of *Locusta*.

ment of the brain and ventral nerve cord from neuroblasts takes place before the extra-embryonic membranes rupture and roll back. During this time, the more lateral embryonic ectoderm pouches out as limb buds and produces a variety of ectodermal invaginations. In due course, after membrane rupture has occurred, the lateral embryonic ectoderm spreads dorsally towards the midline, effecting dorsal closure.

Ectodermal invaginations on the trunk segments give rise to the thoracic and abdominal apodemes, the tracheal system and the genital opening. Invaginations on the head develop as cephalic apodemes and glands. The largest ectodermal ingrowths on the pterygote head are the salivary glands (Fig. 10.5.5), which have been found to originate near the bases of the labial limb buds. When the labial limb buds fuse, the paired openings of the salivary glands come together in the ventral midline to form a median opening on the pharynx. Other ectodermal invaginations on the head (Fig. 10.5.19) form the arms of the tentorium. The anterior tentorial arms originate in the ventrolateral ectoderm between the antennal and mandibular segments. The posterior tentorial arms invaginate from the ventrolateral ectoderm between the maxillary and labial segments. At the same time, between the anterior and posterior pairs of tentorial invaginations, a pair of invaginations at the bases of the mandibles gives rise to the mandibular apodemes. The remaining important structures arising as invaginations of the cephalic ectoderm in the pterygote embryo are the corpora allata. Their origin is closely associated with that of the posterior tentorial arms and has been traced in several species to the region of the mandibular maxillary intersegment.

The further development of the brain and ventral nerve cord proceeds in the same general manner throughout the pterygotes. As the segmental ganglionic masses become more compact and move into the interior of the embryo, they become linked together through the development of neuropile to form the ventral nerve cord. Three pairs of gnathal ganglia, three thoracic pairs and ten abdominal pairs always develop. An eleventh pair of abdominal ganglia has been identified in certain primitive Hemimetabola. In later development, the gnathal ganglia fuse to form the suboesophageal ganglia, and the posterior abdominal ganglia also fuse. The intrasegmental regions of the median strand are known to be incorporated into the segmental ganglia. The development of the antennal and pre-mandibular ganglia follows the same course as that of the ventral ganglia, except that the antennal ganglia move forward in front of the mouth and do not develop a transverse commissure. The commissure connecting the premandibular ganglia remains postanal. At the front of the head, the neuroblasts on either side of the labrum give rise to the paired lobes of the protocerebrum (Fig. 10.5.19) connected by the supraoesophageal commissure. The lateral, optic ganglia of the protocerebrum develop as separate proliferations of the anterolateral ectoderm of the head lobes, without the intervention of neuroblasts.

HATCHING

Like the apterygotes, the pterygote embryos hatch directly from their enclosing membranes. Hatching thus involves the rupture of the chorion and vitelline membrane. The duration of development varies greatly among species, but as a general rule is more prolonged in Hemimetabola.

Development of Paraneoptera and Holometabola

The eggs of Paraneoptera retain most of the characteristics of hemimetabolan eggs, although the smaller insects of this group tend to lay eggs with dimensions less than 1 mm. In one respect, however, paraneopteran eggs are more highly organized than those of the primitive Hemimetabola. A cytoplasmic reticulum enmeshes their yolk spheres and merges at the surface with a distinct, yolk-free periplasm. The same specialization is convergently expressed in the eggs of the Holometabola. Here, too, the dimensions of the egg are generally small, being frequently less than 1 mm. The egg is moderately yolky in generalized orders, but the more specialized like Lepidoptera, Diptera and Hymenoptera show a relative reduction of yolk, a preponderance of the cytoplasmic reticulum and a conspicuous periplasm.

The cleavage is similar to Hemimetabola. The cleavage energids arrive uniformly at the periphery of the yolk mass and merge with the periplasm. Further mitoses then increase the surface population of nuclei, giving a syncitial blastoderm. In the more specialized Holometabola, cytoplasm from the reticulum within the yolk mass also moves out to thicken the syncitial blastoderm. When the nuclear divisions are complete, a simultaneous formation of cell membranes throughout the syncitial blastoderm transforms the later into a cuboidal or columnar, uniform, cellular blastoderm. The columnar condition is characteristic of Diptera and Hymenoptera, the two orders exhibiting the greatest preponderance of cytoplasm relative to yolk in the egg. Differentiation of the uniform cellular blastoderm to a differentiated blastoderm then proceeds. The formation of the embryonic primordium and attenuation of the remainder of the blastoderm as extra-embryonic ectoderm takes place in the Paraneoptera in much the same manner as in the primitive Holometabola, producing a relatively small embryonic primordium. The same process of cellular aggregation in the Holometabola, on the other hand yields a large embryonic primordium, occupying relatively more of the surface of the yolk mass. In the Dermoptera, Psocoptera, Homoptera and certain Coleoptera, a group of rounded cells is separated off at the posterior pole of the embryonic primordium during differentiation of the blastoderm. These pole cells, as they are called, are the primordial germ cells.

PRESUMPTIVE AREAS OF THE BLASTODERM

The cells of the presumptive areas of the uniform blastoderm are thinly spread over the surface of the yolk mass. As blastodermal differentiation proceeds, all except one of these areas lessen greatly in area as the cells become tightly packed and columnar. The exception, of course, is the dorsal, extra embryonic ectoderm, which become more attenuated and spread as blastodermal differentiation proceeds. The majority of Holometabola, in contrast, having a more deeply cuboidal blastoderm, undergo less concentration of cells during formation of the embryonic primordium. The presumptive areas, therefore remain relatively large in area, even at the differentiated blastoderm stage.

The paired bands of presumptive embryonic ectoderm are broader in the Holometabola, especially in the Diptera, Hymenoptera and Lepidoptera, than they are in the hemimetabolan insects.

GASTRULATION

The same mode of entry of presumptive mesoderm into the interior as in the case in Hemimetabola is also seen in the Paraneoptera and in the relatively long embryonic primordia of most Holometabola. In the Hymenoptera and Diptera, in which the presumptive mesoderm is a much broader band of ventral cells, the mode of entry into the interior is substantially modified. In the Hymenoptera, the presumptive mesoderm first separates from the edges of the presumptive ectoderm and begins to sink inwards as a broad plate of cells. Rapid spreading of the presumptive ectoderm towards the ventral midline then completes the process of coverage of the mesoderm. In Diptera, in contrast, the mesoderm becomes internal by invagination. The broad plate of presumptive mesoderm folds into the interior along the ventral midline and closes off as a tube. The edges of the presumptive ectoderm come together in the usual way and the walls of the tube then dissociate and spread out to form the usual single layer of mesoderm beneath the ectoderm. Clearly, the events of mesodermal gastrulation in the Hymenoptera and Diptera are specializations of the basic mode of mesodermal gastrulation in the Holometobala, associated with a broadening of the presumptive mesoderm in both orders. In the higher Hymenoptera and Diptera, the presumptive midgut rudiments can be distinguished at the onset of gastrulation.

The pattern of gastrulation movements and relative cell juxtapositions however, suggests very strongly that the midgut gastrulation movements observed so easily in the Hymenoptera and Diptera, are in fact, standard processes in all pterygotes. The presumptive anterior and posterior midgut cells move into the interior by immigration at the ends of the gastral groove and are overgrown by marginal cells at the surface.

As the gastral groove closes midventrally over the now internal mesodermal and midgut rudiments, the presumptive stomodaeal and presumptive proctodaeal cell lie in the ventral midline at the surface of the embryo, at the two ends of the groove. Without exception, these groups of cells now make independent invaginations, into the interior as short tubes, the superficial openings of which are the mouth and anus respectively (Fig. 10.5.4). Stomodaeal invagination usually follows shortly after closure of the gastral groove, in association with the general lead taken in development by the anterior part of the embryo. Proctodaeal invagination is almost always delayed until the formation of the abdominal segments is well advanced. The Diptera are again an exception. Their abdominal segments are all established as rudiments in the blastoderm and their stomodaeal and proctodaeal invaginations proceed simultaneously, early in gastrulation. As we have seen, the presumptive embryonic ectoderm of pterygote embryos always plays a part in gastrulation, through a ventral spreading movement from either side which closes the gastral groove. Apart from this specialized movement, the presumptive embryonic ectoderm of pterygotes normally resembles that of myriapods and apterygotes in making no gastrulation movements. It already lies in its definitive, organ forming position when formed in the differentiated blastoderm. The extra-embryonic ectoderm also has the usual exclusion from gastrulation movements although it later becomes involved in an elaborate sequence of movements of the germ band relative to the yolk mass.

FURTHER DEVELOPMENT OF THE GUT

The same mode of development of the midgut (Fig. 10.5.5) as observed in the Hemimetabola has also been found in numerous holometabolans with minor functional modifications being noted in the Hymenoptera and Lepidoptera.

DEVELOPMENT OF EXTERNAL FORM

In Holometabola the embryonic primordium is both long and broad, with large head lobes (Figs. 10.5.11 and 10.5.14), although the composition of the head lobes as incipient preantennal and antennal components, and of the post-antennal region as gnathal and thoracic segments and a growth zone (Fig. 10.5.20) is usually similar to the generalized, hemimetabolan condition. The higher Hymenoptera, Diptera and Lepidoptera exhibit further specializations of this condition, the nature of which will be briefly mentioned later.

A similar extension of the posterior part of the growing germ band along the dorsal surface as is the case in Hemimetabola is seen in Holometabola also. During the growth and segmentation of the germ band, limb buds develop on the head and thorax, usually in anteroposterior succession. The antennal

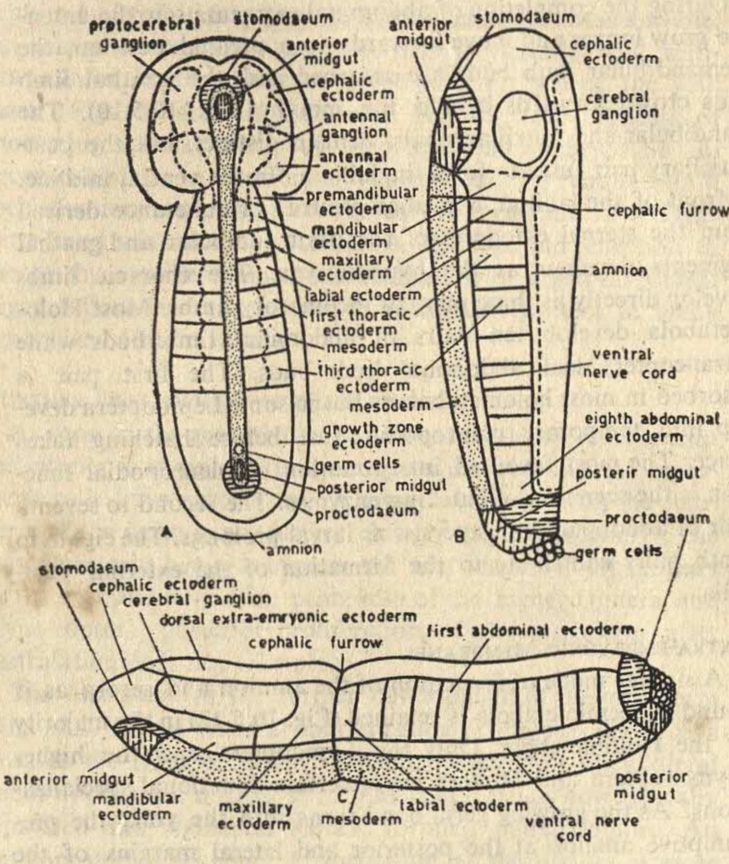


Fig. 10.5.20. Fate maps of pterygote blastoderm.
A—The embryonic primordium of *Tenebrio*.
B—The blastoderm of *Culex*.
C—The blastoderm of *Dacus*.

limb buds arise at the posteromedian corners of the head lobes, behind the level of the invaginating stomodaeum, and are followed by the gnathal and thoracic limb buds in their respective segments. The labrum pouches out midventrally between the head lobes, usually as a single rudiment, but sometimes as a pair of rudiments which later fuse. The paired origin of the labrum is thought to be secondary. Somewhat later than the cephalic limb buds, a transient pair of small, premandibular limb buds develops in front of the mandibles.

During the completion of abdominal segmentation, the antennae grow longer and move forwards to a preanal position, the premandibular limb buds are resorbed and the gnathal limb buds crowd forwards behind the mouth (Fig. 10.5.10). The mandibular and maxillary pairs remain distinct, but the postmaxillary pair fuses to form the labium. In the ventral midline, in front of the labium, a median ventral protuberance derived from the sternal ectoderm of the pre-mandibular and gnathal segments develops as the hypopharynx. The thoracic limbs develop directly as three pairs of locomotory limbs. Most Holometabola develop ten pairs of abdominal limb buds while Paraneoptera lack abdominal limb buds. The first pair is resorbed in most holometabolans but in some Lepidoptera develop into temporary pleuropodia, lost before hatching takes place. The most favoured interpretation of pleuropodial function is the secretion of hatching enzymes. The second to seventh pair of abdominal limbs persist as larval prolongs. The eighth to tenth pairs contribute to the formation of the external genitalia.

EXTRA-EMBRYONIC MEMBRANES

A similar mode of formation of the amnion and serosa as is found in Hemimetabola is retained (Fig. 10.5.12) in the majority of the Holometabola. Only the Lepidoptera and the higher Hymenoptera and Diptera show certain functional specializations. As the growing germ band turns into the yolk, the presumptive amnion at the posterior and lateral margins of the germ band folds over in the usual way to enclose an amniotic cavity, but the cavity remains open to the exterior at the point of inturning. The extra-embryonic ectoderm which will give rise to the serosa does not contribute to the amniotic folds. A delayed formation of anterior amniotic folds finally takes place around the margins of the head lobes and the amniotic cavity is then closed off.

The anterior end of the germ band retains its ventral, anteriorly directed location on the surface of the yolk mass during growth and segmentation. It is usual for the posterior end of the germ band to be extended around the posterior pole of the yolk mass onto the dorsal surface by the time growth and segmentation are completed. When the embryonic membranes rupture and roll back, the germ band does not move as a whole, but contracts strongly in length, so that the posterior end withdraws from the dorsal surface to the posterior pole of the yolk mass (Figs. 10.5.14, 10.5.15 and 10.5.16). This process of shortening, characteristic of holometabolan embryos as an accompaniment to membrane rupture can be required as a specialization consequent on the earlier formation of a large germ band relative to the yolk mass. Rupture and reflection of the emrbyonic membranes and formation of a serosal dorsal organ follow the same course as in the Dictyoptera in many generalized Holometabola.

FURTHER DEVELOPMENT OF THE MESODERM

As soon as the gastrulation movement of the mesoderm into the interior is completed in a pterygote embryo and the mesoderm cells have spread out as a single layer beneath the ectoderm of the germ band, further changes begin. Anteriorly, the mesoderm cells begin to express the first stages of organogeny in the exceptionally long primordia of the higher Diptera and Hymenoptera, posterior proliferation of the mesoderm after gastrulation does not take place.

The first stage in the further development of the mesoderm (Fig. 10.5.12) is a bilateral concentration of the mesoderm cells towards the sides of the germ band, giving two, thick lateral bands and a thin, median strip. The lateral bands now typically separated into segmental blocks, forming paired somites. An anteroposterior sequence of formation of the somites, is more common among the Holometabola. The generalized Holometabola have a similar pattern as is found in Hemimetabola, of somite development, except that the first abdominal somites are small and the residual posterior mass of mesoderm behind the tenth abdominal somites never develops cavities.

It is generally agreed that six pairs of somites is the basic number in the developing pterygote head. The generalized Holometabola confirm this (Fig. 10.5.17), since they too have a small premandibular pair in front of the mandibular pair. In most Holometabola, cephalic somite formation is variously suppressed in favour of a more direct development of cephalic mesodermal structures. The further development of the somites is remarkably uniform throughout the pterygotes (Fig. 10.5.17) which was already described with regard to Hemimetabola.

Gonads and gonoducts: The early segregation of primordial germ cells is followed by a more direct development of the gonads. The germ cells become distinct either during gastrulation or during blastoderm formation, in the vicinity of the posterior end of the presumptive mesoderm or the adjacent presumptive posterior midgut and proctodaeum. Once internal, they segregate as two groups which become embedded in the splanchnic mesoderm strands. A temporary distribution as segmental groups in the abdominal mesoderm has been observed in the Coleoptera, Mesoderm cells cluster around them, completing a pair of compact gonads.

FURTHER DEVELOPMENT OF THE ECTODERM

The pterygote embryonic ectoderm exhibits an early differentiation of neuroblasts as a preliminary to the proliferation of segmental ganglia (Figs. 10.5.12 and 10.5.17). Through teloblastic budding, the neuroblasts proliferate radial rows of small cells inwards as ganglion cells. As the number of cells proliferated by the neuroblasts increases, the radial cell rows merge to form ganglionic cell masses associated with the paired mesodermal somites.

The early development of the brain and ventral nerve cord from neuroblasts takes place before the extra embryonic membranes rupture and roll back. During this time, the lateral embryonic ectoderm pouches out as limb buds and produces a variety of ectodermal invaginations. In due course, after membrane rupture has occurred the lateral embryonic ectoderm spreads dorsally towards the midline, effecting dorsal closure.

The further development of the brain and ventral nerve cord proceeds in the same general manner throughout the pterygotes. As the segmental ganglionic masses become more compact and move into the interior of the embryo, they become linked together through the development of neuropile to form the ventral nerve cord. Three pairs of gnathal ganglia, three thoracic pairs and ten abdominal pairs always develop. In later development, the gnathal ganglia fuse to form the suboesophageal ganglia, and the posterior abdominal ganglia also fuse. In many paraneopterans and some Holometabola, a much greater condensation and fusion of the ventral ganglia takes place.

HATCHING

The pterygote embryos hatch directly from their enclosing membranes. Hatching thus involves the rupture of the chorion and vitelline membrane. In species in which serosal cuticle is secreted, the cuticle appears to be digested by enzymes secreted by the pleuropodia during the later part of embryonic development. Most holometabolous embryos hatch in 10 days or less.

THE BASIC PATTERN OF DEVELOPMENT IN PTERYGOTES

In spite of their immense variability, the pterygotes reveal their standing as a unitary group by the ease with which all of their modes of embryonic development can be related to a single common theme. Much of this theme is further shared in common with the apterygotes and in particular, with the Thysanura, although there are some significant differences.

Pterygote development begins with the formation of a uniform blastoderm covering a yolk mass which contains vitellophages, within the blastoderm, the presumptive areas centre on a narrow band of presumptive mesoderm cells, in the ventral midline. The presumptive embryonic areas are broadly spread at this stage, but become concentrated together to form an embryonic primordium, with associated attenuation of the presumptive extra-embryonic ectoderm, before gastrulation begins.

The presumptive midgut comprises two small areas, also in the ventral midline, as anterior midgut area in front of the presumptive mesoderm and a posterior midgut area behind the presumptive mesoderm. These areas are distinct from the vitellophages within the yolk, which arise during cleavage and blastoderm formation but do not subsequently contribute to the formtion of the midgut epithelium. The presumptive stomodaeum lies as an arc around the anterior face of the presumptive anterior midgut. The presumptive proctodaeum forms a small area in midline behind the presumptive posterior midgut.

Lateral to the midventral area, and more broadly spread around the anterior end of the lateral, is a bilateral band of presumptive embryonic ectoderm, with a marginal zone of presumptive preantennal and antennal ectoderm. The narrower, post-antennal bands consists of the presumptive premandibular, gnathal and thoracic segmental ectoderm, followed by growth zone ectoderm. When blastodermal differentiation has occurred, the embryonic primordium occupies the ventral midline of the surface of the yolk mass and is nearer to the posterior pole than to the anterior pole.

Gastrulation takes place by a midventral immigration of the mesoderm and midgut rudiments, accompanied by the temporary formation of a gastral groove and followed by closure of the groove through the median approximation of the embryonic ectoderm. The midgut rudiment now lie internal to the presumptive stomodaeum and proctodaeum respectively. The mesoderm spreads out as a layer beneath the embryonic ectoderm. Within this layer, the cells of cephalic and thoracic mesoderm are already present, but the abdominal mesoderm is only represented by a posterior growth zone associated with the ectodermal growth zone.

As growth zone activity proceeds, the embryonic primordium elongates and undergoes segmentation, then limb bud formation. Increase in length is accompanied by a forward shift of the anterior end of the germ band along the ventral midline of the yolk mass, and later by a downward flexure of the posterior end of the abdomen at the posterior pole of the yolk mass. This is the only vestige of dorsoventral flexure. Early in elongation and segment formation the presumptive amnion folds ventrally over the surface of the germ band, carrying the edge of the extra-embryonic ectoderm with it, and merges in the midline to complete the amnion and external serosa. The amnion stretches as elongation of the germ band continues.

The segmenting germ band develops distinct mandibular, maxillary and postmaxillary segments behind the head lobes, followed by three thoracic segments and eleven abdominal segments. A small premandibular segment later becomes transiently distinct between the head lobes and mandibular segment. The head lobes do not develop any intersegmental grooves, but the labrum arises in front of the mouth and the antennae grow out at the posteromedian corners of the head lobes, behind the mouth. Mandibular, maxillary and labial limb buds arise, followed by three thoracic pairs, eleven abdominal pairs and a small, transient, premandibular pair. In later development, the antennae become preoral, the gnathal limb buds crowd forwards behind the mouth, the postmaxillary pair fuse to form the labium and the thoracic pairs develop as locomotory limbs. The first seven and the tenth pair of abdominal limb buds are resorbed before hatching, but the eighth and ninth pairs contribute to the external genitalia and the eleventh pair develops as the cerci.

The amnion and serosa remain intact until segmentation and limb-bud formation along the germ band are complete, but then fuse, rupture and roll back to expose the embryo once more at the surface of the yolk mass. The serosa contracts into a small mass, the serosal dorsal organ, on the antero-dorsal surface of the yolk mass, and is subsequently resorbed into the yolk. The amnion replaced the serosa as a provisional membrane at the yolk surface, but is gradually contracted and resorbed into the yolk as the definitive body wall grows dorsally to replace it. During later growth and development after rupture of the extra-embryonic membranes, the germ band extends forwards to reach the anterior pole of the yolk mass, as well as upward towards the dorsal midline. The forward flexure of the posterior end of the abdomen persists until hatching.

Underlying the changes sunmarized above, the further development of the mesoderm begins with posterior proliferation at the growth zone, accompanied by a bilateral segregation of the mesoderm into paired bands separated by a thin layer of median mesoderm in the ventral midline. The median mesoderm gives rise to haemocytes, except in the antennal segment and preantennal part of the head, where it develops as stomodaeal musculature and as labral and other cephalic muscles. The mesodermal bands break up into paired somites which develop coelomic cavities. The labial, thoracic and first abdominal somites are large, the second to tenth abdominal somites small, but all follow the same mode of development. An appendicular lobe grows into the associated limb bud and gives rise to intrinsic limb musculature, except in those abdominal segments in which the limbs are resorbed. A dorsolateral lobe grows towards the dorsal midline. Cardioblasts at the dorsal margin of the dorsolateral lobe contribute to the heart. The somatic wall of the same lobe develops as the dorsal longitudinal muscle, fat body, pericardial floor and associated structures. The somatic wall of the ventral part of the somite develops as ventral longitudinal muscle, extrinsic limb muscle and fat-body. The splanchnic walls of the somites separate off and join up to form paired splanchnic mesodermal strands associated with midgut strands. The musculature of the midgut arises from these strands. Germ cells become associated with the splanchnic mesoderm of certain abdominal somites and give rise to the gonads. The gonoducts are formed from coelomoduct outgrowths of the splanchnic mesoderm of certain posterior abdominal somites. Behind the tenth abdominal segment, a posterior mass of mesoderm associated with the proctodaeum develops a pair of temporary coelomic cavities, but later spreads over the proctodaeum and gives rise to proctodaeal musculature.

In front of the labial segment, somite development is more specialized. The maxillary and mandibular pairs are small and develop as limb musculature only. The small premandibular pair gives rise to the suboesophageal body and perhaps contributes to the stomodaeal musculature. The antennal pair, which originate post-orally but subsequently become pre-oral are large and retain a typical appendicular lobe, but otherwise develop as fat body and, at their dorsal margins, anterior aorta. A vestigial pre-antennal pair of somites is formed and contributes to the labral musculature.

The embryonic ectoderm proliferates posteriorly and becomes segmentally delineated in association with the mesoderm. Ventrally on either side of the midline from the mandibular to the eleventh abdominal segment, the ectoderm differentiated in part as neuroblasts from which the ganglia of the ventral nerve cord are proliferated. The neurilemma arises from ganglion cells. Along the ventral midline, the ectoderm forms a median strand of tall cells. The intrasegmental parts of the median strands are incorporated into the ganglia, but fate of the intersegmental parts is still not clear. Ventrolaterally, the segmental ectoderm pouches out as limb buds. Laterally, the ectoderm gives rise mainly to hypodermis, gradually spreading towards the dorsal midline as dorsal closure proceeds.

In front of the mandibular segment, paired premandibular (tritocerebral) and antennal (deutocerebral) ganglia develop from neuroblasts. The premandibular ganglia retain a post-oral tritocerebral commissure but the antennal ganglia become preanal and are not connected by a transverse commissure. The pre-oral protocerebral ganglia develop in part from neuroblasts, but the lateral, optic lobes of the protocerebrum arise as proliferations of the lateral ectoderm of the head lobes, without the intervention of neuroblasts.

Paired, segmental, ectodermal invaginations on the trunk give rise to the tracheal system and apodemes. Ectodermal invaginations on the head give rise to the labial salivary glands, anterior and posterior tentorial arms, mandibular apodemes and corpora allata. The anterior tentorial arms originate ventrolaterally in the region of the antennal mandibular intersegment. The posterior tentorial arms and corpora allata arise in the region of the maxillary labial intersegment.

In the further development of the gut, the stomodaeum develops as the epithelial lining of the foregut and also buds off the ganglia of the stomatogastric system from its dorsal midline. The proctodaeum develops as the epithelial lining of the hindgut and proliferates Malpighian tubules at its free-end. The anterior and posterior midgut rudiments attached to the free ends of the stomodaeum and proctodaeum respectively, proliferate paired midgut strand which flank the yolk mass and meet in the midregion of the embryo. The paired midgut strand then spread to enclose the yolk mass and form the lining epithelium of the midgut. The yolk and vitellophages are digested.

DEVELOPMENT OF THE HEAD

Externally, as we have seen, the head rudiments comprise a pair of rounded head lobes with posteromedian antennal rudiments, a pre-mandibular segment region and three gnathal segments. Internally lie paired preantennal, antennal, pre-mandibular and gnathal somites. As in the Thysanura, the antennal somites contribute the anterior aorta and the premandibular somites develop as the suboesophageal body.

Invagination of the stomodaeum, overgrowth of the labrum and preoral migration of the antennal rudiments takes place, as they do in the apterygotes, while the segments behind the antennal segment are still well spread out along the ventral midline. Mandibular, maxillary and labial limb buds develop and conspicuous premandibular limb buds are also transiently developed during the forward migration of the antennae. The mouth parts then crowd forwards around the mouth and the labial limb buds unite to form the labrum. Posterior tentorial arms develop as ectodermal invaginations associated with the labium, while labial salivary glands also arise as ectodermal invaginations on the labial segment. Meanwhile, the head lobes have developed the protocerebral ganglia, partly from neuroblasts. The antennal, premandibular and gnathal ganglia also develop from neuroblasts and the gnathal ganglia merge to form the suboesophageal ganglion. Recent evidence that a pair of segmental preantennal ganglia can be distinguished within the protocerebral complex supports the argument that a vestigial preantennal segment is incorporated into the pre-antennal part of the pterygote head, though most of this region is still best interpreted as a large pre-segmental acron.

The basic pattern of development of the pterygote head is thus similar in all respects to that of the apterygote hexapods and finds its closest comparison among the Thysanura. These findings lend further weight to the view that the Pterygota are more closely related to the Thysanura than to the other apterygote classes. They also indicate that all extant hexapods, including the Pterygota, diverged from an ancestral stock which was already labiate. It should, therefore, be possible to elucidate a basic theme for hexapod development, through which the hexapods can be compared with the myriapods and Onychophora.

6. ARACHNIDA

Holm (1954) investigated the development of an arthognath spider, *Ischnothele karschi* and Yoshikura (1954, 1956) has studied the development of *Heptathela kimurai* which belongs to the group Liphistudae. The works of these two investigators are considered to have made significant contributions to the general knowledge of spider embryology. Some methods, the knowledge of which is essential in studying the embryology of spiders are described below.

The chorion of the spider egg is an opaque membrane which obscures the interior of the egg under ordinary conditions. The processes of development going on inside the chorion can easily be followed with a binocular microscope, if the eggs are immersed in liquid paraffin or glycerine. As fixatives for spider eggs, caronoy's fluid, alcohol-Bouin's and Petrunkevisch's fluids are used. It is difficult to obtain good sections through the early stages because of the large amount of yolk. To overcome this, the embryos are overstained with borax carmine for five to six days, differentiated slowly with 1% acid alcohol and then sectioned. Good results have been obtained by using toluol in the embedding process. The paraffin block is cut until the embedded material is exposed at the cut surface and then immersed in water for one day before sectioning. For total observation of the embryo, the fixed embryo is readily and satisfactorily stained with Ehrlich's hematoxylin.

Structure of Eggs

The eggs are usually deposited together with a drop of fluid on a specially prepared mat of silk, and covered with more threads to form a cocoon or egg sac. The fluid evaporates rapidly, leaving crystalline particles on the surface of the egg. The eggs are usually spherical or oval, measuring between 0.5 to 1.8 mm. in diameter. The egg is covered by two membranes, the chorion secreted by the oviduct and the vitelline membrane, secreted by the egg itself (Fig. 10.6.1). The irregular surface of the chorion makes the membrane opaque and the vitelline membrane is a thin, elastic, transparent membrane. Within the vitelline membrane is a large yolk mass consisting of small, globular yolk granules and large irregular yolk bodies for-

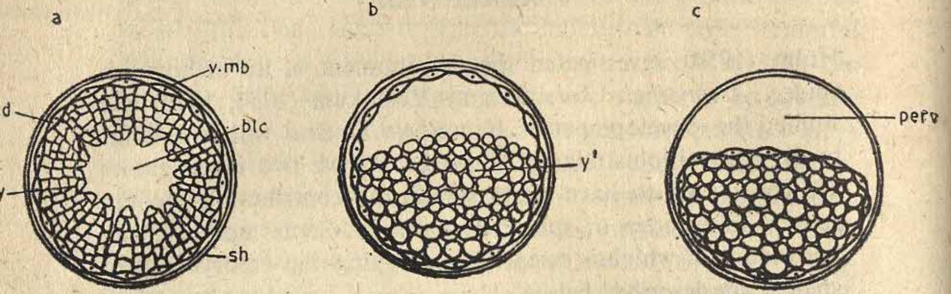


Fig. 10.6.1. Schematic stretches of longitudinal sections of spider eggs, showing egg structure and contraction of blastula.
ble—blastocoel; bld—blastoderm; perv—perivitelline space; sh—shell; v.mb—vitelline membrane; y—yolk granule; y′—yolk mass segregating from blastoderm.

med of accumulation of yolk granules. The eggs immediately after deposition have only yolk granules but within a few hours the yolk bodies appear, especially at the under side of the egg.

At the time of oviposition the nucleus is situated between the centre and the circumference of the egg. After completion of the reduction division, the pronucleus migrates to the centre of the yolk mass. The cytoplasm consists of the centroplasm, which contains the egg pronucleus, the periplasm, which envelopes the yolk mass and the protoplasmic reticulum which connects the centroplasm with the periplasm through the yolk mass, forming a complicated delicate network between the yolk granules.

FERTILIZATION, CLEAVAGE AND BLASTODERM FORMULATION

The sperm and the egg pronuclei unite three or four hours after oviposition. The tempo of subsequent developmental processes differs according to species and temperature. The following description will be based on the egg of *Agelena labyrinthica* developing at a temperature of 22°C. In this spider the first nuclear cleavage takes place 6 hours after oviposition. As the subsequent cleavages occur, the nuclei, surrounded by cytoplasm derived from the cytoplasmic reticulum, migrate slowly between the yolk bodies to the surface of the egg. Sixteen hours after oviposition, the nuclei, their number increased to 8, have reached a position midway between the centre and the periphery of the egg. The segmentation of the yolk accompanies these nuclear cleavages. The yolk bodies arranged radially from centre to periphery compose yolk columns, and several of these join to make a yolk pyramid. At the centre of the egg, a small cavity, enclosed within the central ends of the yolk pyramids, forms a blastocoel, which enlarges as the yolk is consumed (Fig. 10.6.2). At the 64 nucleus stage (after 6th cleavage) the nuclei approach the surface of the egg, and the cytoplasm enveloping each nucleus extends pseudopodia and established connection with the periplasm. Eventually the nucleii reach the surface of the egg. At the surface the nuclei continue to cleave once or several times, while they form a layer and envelop the surface of the egg. This layer is the blastoderm, and with its completion 35 hours after oviposition, the embryo enters the blastula stage.

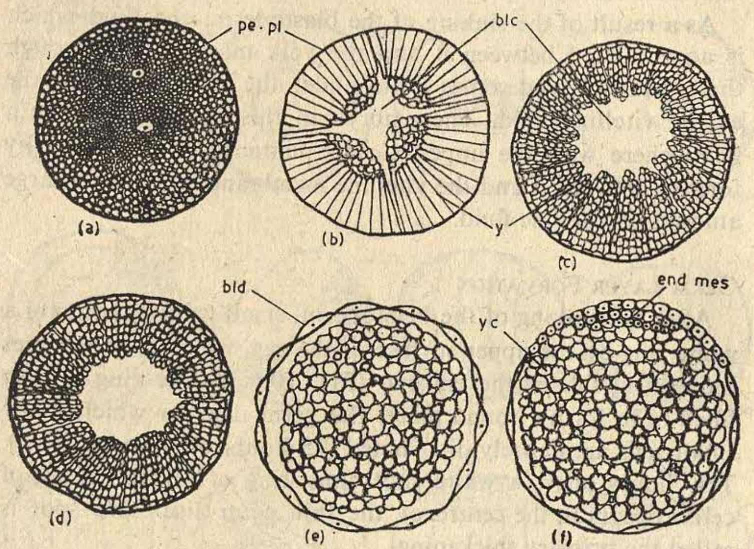


Fig. 10.6.2. Cleavage and blastulation in the spider *Theridium maculatum*.
blc—blastocoel; bld—blastoderm; end. mes—endo-mesoderm cell; pe. pl—peripheral cytoplasm; y—yolk pyramid; ye—yolk cell.

Soon after the formation of the blastoderm, the whole yolk mass contracts, and the yolk granules of the upper side of the egg separate from the blastoderm and sink downward, obliterating the blastocoel. Accompanying this contraction, the fluid of the blastocoel permeates between the blastoderm and the yolk surface. The contraction is due to the shrinkage of the protoplasmic reticulum between the yolk pyramids. The blastoderm then begins to contract, separates from the vitelline membrane and sinks slowly to the surface of the yolk mass (Fig. 10.6.1).

As a result of the sinking of the blastoderm, the fluid which is accumulated between it and the yolk mass passes through the blastoderm and comes to lie under the vitelline membrane as the vitelline fluid. The embryo in this stage appears as a hemisphere with the upper surface flattened and the cavity formed between it and the vitelline membrane contains a large amount of vitelline fluid.

GERM LAYER FORMATION

After the sinking of the blastoderm, small cells gather into a white ring on the upper surface of the egg, which later becomes the ventral side of the embryo (Fig. 10.6.3). The ring is then filled in to form a round plate, the germ disc, in which many small cells are densely distributed (75 hours after oviposition). Ten hours later, a white spot consisting of several layers of cells appears in the centre of the thin germ disc. This spot is called the primary thickening.

A shallow groove of irregular shape which appears in the centre of this thickening is the blastopore. According to the results of local vital staining, most of the cells forming the early germ disc invaginate inward through the blastopore, and spread along the inner side of the germ disc. The cells which invaginate early become endoderm, whereas those invaginating later become mesoderm. The process is therefore a form of gastrulation, which result in the formation of the primary thickening. As development proceeds, the germ disc spreads to cover the whole upper surface of the yolk mass. The primary thickening at first does not extend above the egg surface, but within a short time one part of it rises to form a hemispherical protuberance (100 hours after oviposition) which separates from the primary thickening and migrates toward the margin of the germ

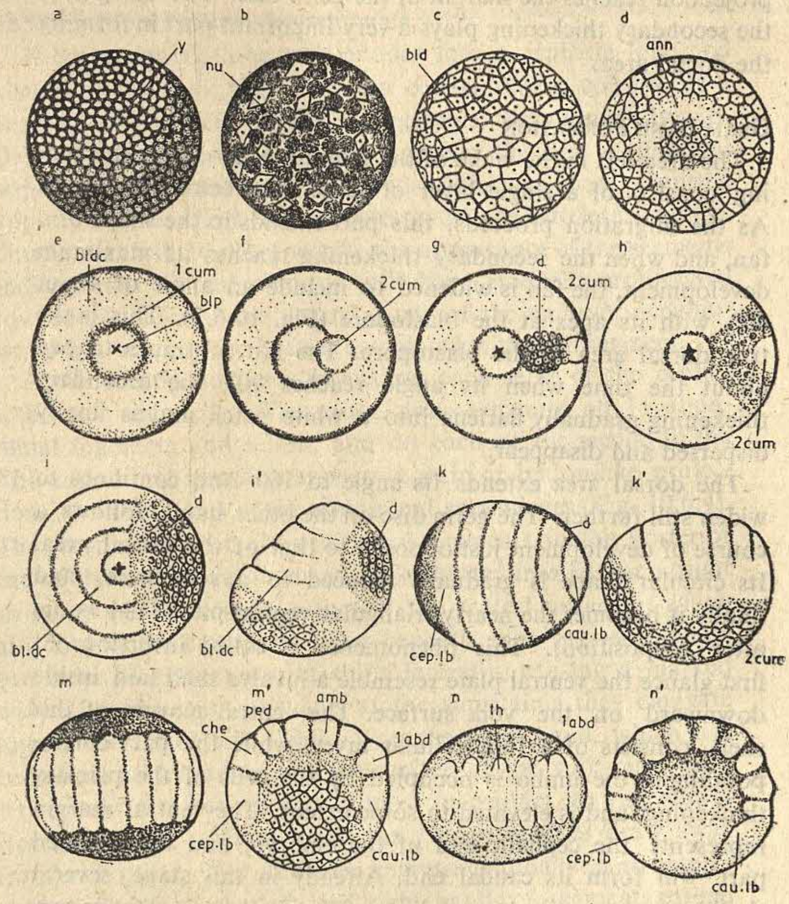


Fig. 10.6.3. Stages in the development of *Agelena*.
1 abd—1st abdominal segment; 1 cum—primary thickening, 2 cum—secondary; amb—ambulatory leg; ann—annular zone; bld—blastoderm; bl.de—blastodisc; blp—blastopore; cau. lb—caudal lobe; cep.lb—cephalic lobe; che—cheliceral segment; d—dorsal area; nu—cleavage nucleus at surface; ped—pedipalpal segment; th—thoracic extremity appendage; y—yolk granule.

disc (Fig. 10.6.3). This protuberance is called as a *secondary thickening*. As this secondary thickening migrates away from its original position, its height increases steadily, attaining a maximum about 120 hours after oviposition, when the centre of the projection reaches the margin of the germ disc. The margin of the secondary thickening plays a very important part in forming the dorsal area.

GERM BAND FORMATION

The area left in the wake of the migrating secondary thickening consists of a single layer of large, flat, ectodermal cells. As the migration proceeds, this part extends in the shape of a fan, and when the secondary thickening reaches its maximum development, the fan is widened to include an angle of about 60°, with its apex at the blastopore (Fig. 10.6.3). This is the true dorsal area of the blastopore. The fan continues to pen about the time when its angle reaches 90°, the secondary thickening gradually flattens into a white patch and is finally dispersed and disappear.

The dorsal area extends its angle to 180° and continues to widen still further. The germ disc on the other hand, follows a course of development just opposite to that of the dorsal area. Its circular shape is gradually reduced to a semicircle, and finally it becomes the nearly triangular ventral plate (144 hours after oviposition). This phenomenon is called inversion. At first glance the ventral plate resemble a bivalve shell laid inside downward on the yolk surface. The ventral margin of this shell consists of a thin cellular layer, while the part corresponding to the umbo is occupied by the cells of the primary thickening, and is elevated to some extent. The ventral margin represents the cephalic end of the embryo, and the elevated part will form its caudal end. Already in this stage, several shallow grooves, like growth lines of the shell, somewhat vaguely indicate 3-4 incipient segments. As these segments become definite, the posterior parts of the embryo also widen, and the embryo takes the form of a germ band which covers the upper surface of the egg. Before long, the foremost segment of this germ band becomes a semicircular procephalic lobe, and the primary thickening of the caudal end forms a caudal lobe, which is pushed around to the undersurface of the egg as the number of segments increases. Five segments are recognizable between these two lobes at this stage (155 hours after oviposition). The segment nearest to the procephalic lobe is the pedipalpal segment; posterior to this are the first, second, third and fourth ambulatory leg segments.

The procephalic lobe next divides into a cephalic lobe and cheliceral segment, so that the cephalothorax consists of a cephalic lobe and six subsequent segments. Simultaneously with the formation of the cheliceral segment, the first abdominal segment is separated from the caudal lobe. As these segments appear, the mesoderm differentiates under the ectoderm in coordination with it. In other words, the appearance of the segments indicate that the mesodermal cells have gradually proliferated toward the anterior and ventral parts of the embryo, accumulating into rectangular masses associated with each segment.

A pair of appendages is formed on each setae of the cephalothoracic segments. The caudal lobe separates off 11 abdominal segments and telson, and on each of the second to the fifth abdominal segments appears a pair of button-like projections, the rudiments of the abdominal appendages (Fig. 10.6.4). The germ band, from the pedipalpal segments to the posterior end of the abdominal segments, is separated into left and right strips by a shallow median furrow, the ventral surface of which also divides the mesoderm lying beneath each segment. These two strips of segments next shifts laterally, causing a marked widening of the ventral sulcus at the same time they elongate toward the dorsal side. This causes the germ band, which has been bent backward around the surface of the yolk mass with its cephalic and caudal ends almost in contact, to rise slowly from the yolk surface, while the two extremities draw away from each other. The abdomen migrates gradually in an anteroventral direction, the left and right bands of segments extend around the side to reach the dorsal median line, and then they also extend around the sides to reach the dorsal median line, and then they also extend ventrally and almost completely surround the yolk mass. The embryo then finally becomes bent forward and this phenomenon which is regarded as a specific feature of developmental process in spider eggs is called reversion.

PRESUMPTIVE REGION OF SPIDER EGG

Holm (1952) studied in detail the cellular migration on the surface of the *Agelena* egg by the method of local vital staining

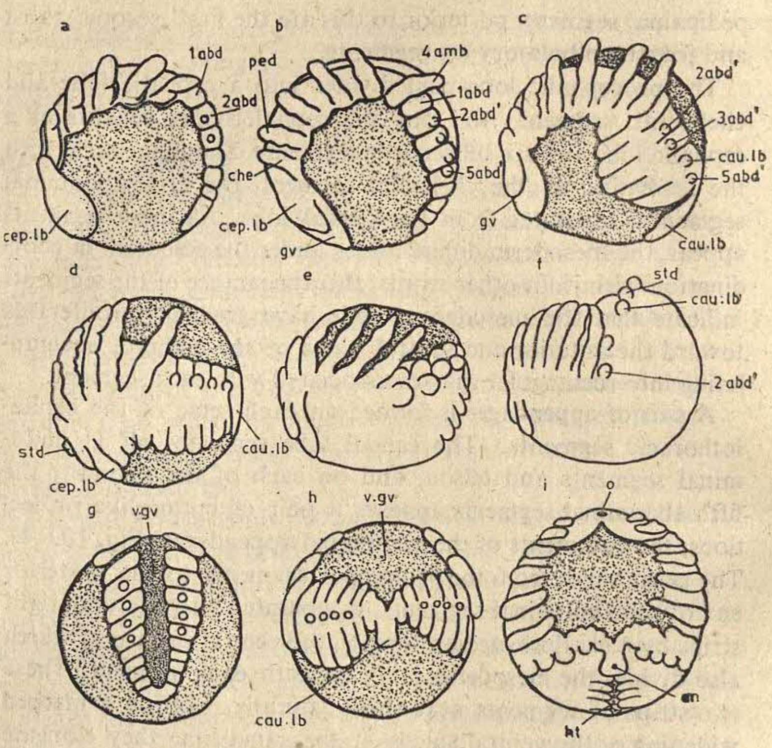


Fig. 10.6.4. Reversion of *Agelena* embryo.
1 abd—first abdominal segment.
2 abd′—second abdominal extremity appendage.
3 abd′—third abdominal extremity appendage.
4 amb—4th ambulatory leg.
5 abd′—5th abdominal extremity appendage.
an—anus; cau—lb—caudal lobe; cep.lb—cephalic lobe; che—cheliceral segment; gv—cerebral groove; ht—head; o—mouth; ped—pedipalpal segment, std—upper wall of stomodaeun, v.gv.—ventral groove.

and mapped the presumptive organ-forming regions. According to him a striking cellular migration toward the blastopore occurs after blastoderm formation in *Agelena* and the germ disc surrounding the blastopore does not represent the rudiment of the embryo as in most animals, but is the part which will invaginate through the blastopore and later become the mesoendoderm, while the future ectoderm takes its origin from the blastular cell layer at the periphery of the germ disc. The so-called germ disc in this stage, does not correspond to the usual germ disc, and the region surrounding it does not represent an extra-embryonic area as in other animals. The true-extra embryonic area of the spider embryo appear only after the embryonic dorsal region has been formed by the separation of the secondary thickening.

Holm was not able to determine the boundary between endoderm and mesoderm, but as the stained cells which make up the secondary thickening later appear in the intestinal wall and constitute the bulk of the abdominal endoderm, he concluded that the cells in the anterior half of the primary thickening which invaginate first similarly become the endoderm of the cephalo-thorax, while the cells invaginating lateral form the mesoderm. These results are incorporated in Fig. 10.6.5 which represents the presumptive map of the early gastrua stage. The true germ disc at this stage is seen to consist of the part of the presumptive secondary thickening which spreads out over the prospective dorsal area together with the remaining area of presumptive mesoectoderm surrounding it. The germ disc is enclosed within an area of presumptive ectoderm. In this stage,

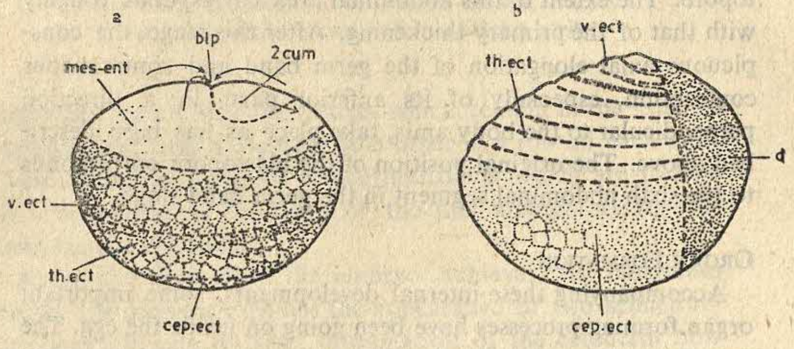


Fig. 10.6.5. Map of presumptive regions in the *Agelena* egg.
a—embryo at gastrulation.
b—embryo just after completion of invagination.
2 cum—presumptive area of secondary thickening, which invaginates through blastopore; blp; cep. ect—presumptive area of cephalic ectoderm; mes. ent—presumptive area of mesentoderm; th.ect—presumptive area of thoracic ectoderm; v.ect—presumptive area of ventral ectoderm.

the boundary between the presumptive mesoectoderm and presumptive ectoderm corresponds roughly to the edge of the germ disc. The blastodermal layer outside the germ disc is divided into three areas; the cephalic ectoderm, which lies on the opposite side of the egg, farthest from the blastopore; an annulate area of thoracic ectoderm surrounding the former, and the abdominal ectoderm, which lies between the germ disc and the thoracic ectodermal area.

The margin of the germ disc corresponds roughly to the boundary between the cephalic lobe (Fig. 10.6.5) and the anterior margin of the thoracic cheliceral segment. Next to the cheliceral segment, the pedipalpal segment and ambulatory leg segments are arranged in concentric areas, and posterior to these there is an abdominal region also consisting of concentric areas which have as their centre a point slightly posterior to the blastopore. The extent of this abdominal area corresponds roughly with that of the primary thickening. After this stage, the conspicuous axial elongation of the germ band and simultaneous contraction, especially of its anterior part, in a direction perpendicular to the body amis, take place as has been described above. The original position of the blastopore corresponds to the sixth abdominal segment in the germ band stage.

ORGAN FORMATION

Accompanying these internal developments, some important organ forming processes have been going on inside the egg. The mesoderm underlying the ectoderm of each segment increases independently to form a coelomic sac. As the reversion of the embryo proceeds, these sacs grow laterally to enwrap the yolk mass from the sides. The cavities of the coelomic sacs later disappear and their walls become muscles while their ends which extend from the two sides toward the back of the embryo grow to reach the dorsal median line. There they meet to form the heart in the abdominal region and dorsal aorta, continuous with the heart, in the cephalothoracic region (Figs. 10.6.4 and 10.6.6).

Each coelomic sac which has developed in a segment bearing appendages forms a projection into a base of the appendage; these cavities also disappear in time and the tissues transform into muscles. However, in the four segments bearing the ambu-

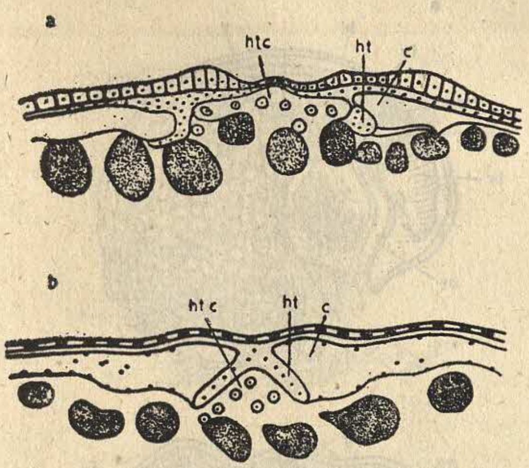


Fig. 10.6.6. Development of heart in *Agelena* embryo. c—coelomic sac; ht—heart wall; ht.e—heart cavity.

latory legs, small saccular vestiges which remain in the base of each segment later unite with each other in a row on each side. These open into a pair of ectodermal ducts which are formed by invagination at the bases of the first ambulatory legs, to form the coxal glands.

About the time when the embryo achieves its maximum length, the cephalic lobe has the appearance of two semicircular plates laid side by side; invagination of the ectoderm now takes place along their margins, forming the cerebral grooves (Fig. 10.6.4). The grooves become deeper, and their walls thicken and acquire a complex structure to form the main parts of the brain or supra-oesophageal ganglia. On the other hand, paired ectodermal thickenings appear on the two sides of the median ventral line, in each of the segments lying posterior to the cephalic lobe. These thickenings later separate from the ectoderm, become connected with each other by a nerve cord, and form the ventral nerve chain. In time the ganglion of the cheliceral segment unites with the supra-oesophageal ganglion, while the ganglia of the other segments migrate forward as development proceeds and come together into a mass in the cephalothorax before hatching, as the sub-oesophageal ganglion (Fig. 10.6.7).

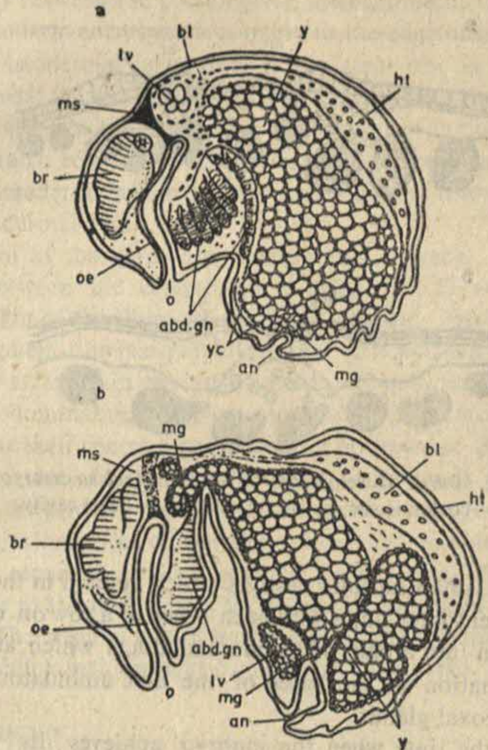


Fig. 10.6.7. Saggital sections through the embryos of the spider, *Theridion maculatum* in succeeding stages of organ formation, showing migration of ganglia and formation of digestive tract.

abd. gn—abdominal ganglion; an—anus; bl—blood corpuscles; br—brain, ht—heart; lv—liver; mg—midgut; ms—muscle; o—mouth, oe—oesophagus; y—yolk granule; y.e.—yolk cell.

The eyes of spiders consists of a pair of central eyes and three pairs of lateral eyes. The central eyes are formed first, behind the cephalic lobes near the median ventral line, as invaginations of the ectoderm which are closely connected with the brain. As the result of complicated process of infolding, the ectoderm becomes double layer and differentiates into a lens and retina (Fig. 10.6.8). The lateral eyes appear in the dorso-lateral regions of the cephalic lobes as simple, ring-like hollows of the ecto-
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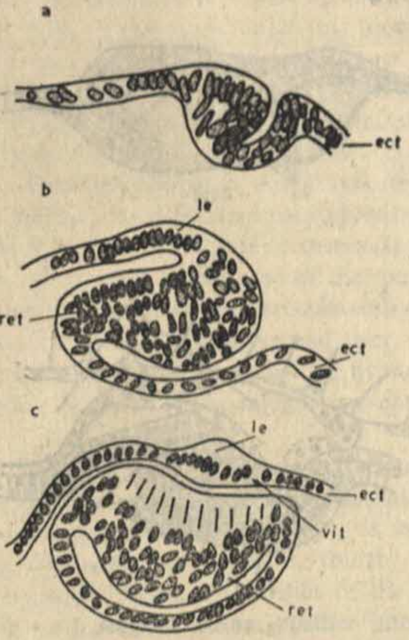

Fig. 10.6.8. Development of central eye in *Agelena* embryo. ect—ectoderm; le—lens; ret—retina, vit—vitreous body.

derm; they differ from the central eyes in that the bottom of each hollow is transformed directly into the retina.

At about the time when the reversion of the embryo occurs, marked changes also take place in the abdominal appendages. Those of the second and third abdominal segments become flattened, and ectodermal invaginations appear in the posterior parts of their bases. These are the rudiments of the lung sacs; in the inner wall of each sac arise many infolds which develop into the book-lungs (Fig. 10.6.8). Two pairs of such book lungs are formed in the tetrapnaumon spiders. In *Agelena*, however, only a single pair of these structure is formed in the second abdominal segment; while the invagination of the appendages on the third abdominal segment became tracheae (Fig. 10.6.9).

Each appendage of the fourth and fifth abdominal segments divides into an endopodite and exopodites. In *Agelena*, the paired endopodites of the fourth segment fuse and change into

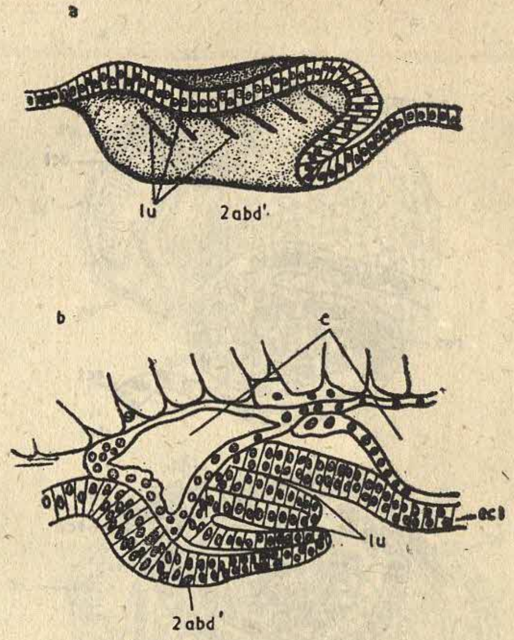

Fig. 10.6.9. Lung sac.
a—inner side, viewed from posterior side.
b—cross section.
2abd'—2nd abdominal extrevity appendage; c—coelom ic sac; ect—ectoderm, in—infolding of lung sac wall.

the *colulus*, which does not perform the function of spinning. The exopodites of this segment become the anterior spinnerets, the endopodties of the fifth segment form the intermediate spinnerets, and the exopodites the posterior spinnerets. The ectoderm of the tips of these spinnerets invaginates and differentiates into the spinning glands.

As the cerebral grooves are deepening, a stomodaeum is formed by the invagination of the ectoderm on the median line behind the cephalic lobes and just anterior to the base of the palpi. This extends inwards and later differentiates into a pharynx, an oesophagus, and a sucking stomach. By means of this large blind sac, which sends branches into each pair of legs, the spider is able to generate the negative pressure necessary to suck the body fluids from its prey. Shortly after the appearance of the stomodaeum, a proctodaeum is formed by the invagination of the ectoderm in front of the caudal lobe; this becomes the rectum. These ectodermal parts of the digestive tract at the anterior and posterior ends of the embryo are connected by the midgut. The developmental history of this structure is complex, but in general is regarded as being derived from the endoderm. Posteriorly a sheet-like accumulation of yolk cells appear near the caudal lobe; this soon become the sterocorpal packet and is connected with the rectum. A pair of anteriorly directed Malpighian tubes are formed at the sides of the sterocorpal pocket. Another accumulation of endodermal cells also occurs posterior to the stomach; a little later these spread over and enwrap the yolk, which has by this time been divided by mesodermal septa into four pairs of blocks. The midgut is eventually formed in this region.

In the spiders, the germ cells can be recognized only in a relatively late stage, when germ-band formation is complete and the embryo is undergoing reversion, as large cells in the wall of the coelomic sacs of third, fourth, fifth and sixth abdominal segments. When the cavities of the sacs disappear, these cells in each segment come together into lateral clumps which give rise to the genital glands. The coelomic sacs of the second abdominal segment elongate and connect with ectodermal invaginations to form the genital ducts. These processes by which the reproductive organs develop are quite similar to those which give rise to the coxal glands (Fig. 10.6.10).

Hatching and larvae: The embryo break open its chorion, along a line connecting a pair of egg-teeth which are formed on the outer side of the bases of the palpi, and escapes from it by expanding and contracting movements of its body. The egg teeth are shed during this process and remain stuck to the discarded vitelline membrane. The hatched larvae generally leave the nest after moulting once within the egg sac. Spiders usually attain their full growth after moulting six to eight times.

DEVELOPMENT IN OVOVIVIPAROUS ARACHNIDS

As an example of ovoviviparous species (Buthidae) we can take *Euscorpius italicus*. The egg of this species is elongate, ovoid and is about 400 μ long when released into the oviduct, although the embryo increases in size during later develop-

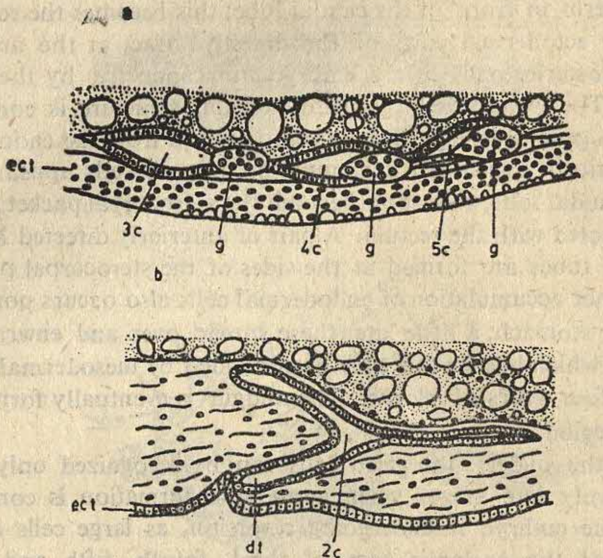

Fig. 10.6.10. L.S. of part of *Agelena* embryo at reversion stage. 2e, 3e, 4e, 5e—2nd, 3rd, 4th and 5th abdominal coelomic sacs; dt—gonadal duct, growing toward ectoderm. ect—ectoderm; g—masses of germ cells appearing in coelomic wall of each abdominal segment.

ment. The yolk-free cytoplasm is in the form of polar cap at one end and the zygote nucleus lies close to this pole. At the first cleavage division, the polar cap furrows across the middle and become two flattened, equal cells. The second cleavage division proceeds in the same way, perpendicular to the first. As division continue, the cells become smaller and the cap of cells spreads more extensively over the surface of the yolk mass. There is no evidence to suggest that any cleavage furrows pass into or through the yolk mass. Eventually, the spreading cells extend to cover the entire surface of the yolk mass, but the onset of gastrulation and further development is observed when the polar cap is still restricted to about 64 cells. The polar cap of cells is actually a precociously formed embryonic primordium. As the embryonic primordium begins to increase in length and gives rise to a germ band, the attenuated blastoderm separates from the margin of the primordium and extends to merge over the outer surface of the primordium. As a result, the yolk mass and embryonic primordium lie within a thin external envelope of cells, customarily called the serosa. It persists until birth and is sloughed off after birth. Presumably the serosa has a functional role in relation to the development of the embryo within the maternal oviduct, but nothing is known of this function at the present time.

In addition to the serosa, buthid embryos also form a second embryonic membrane, the amnion. The membrane arises during the early elongation of the germ band. The margin of the germ band turns downwards and spreads inwards from all sides to merge along the ventral midline. The amnion thus encloses an amniotic cavity beneath the external surface of the germ band, but the functional significance of the membrane and cavity are again unknown. During elongation of the germ band and subsequently during closure, the amnion continues to cover the external surface of the embryo, so that it eventually form a second complete envelope beneath the serosa. Like the serosa, the amnion is shed only after birth.

We can now follow the development of the buthid germ band itself (Fig. 10.6.11). There is no evidence at present to indicate that a gastral groove is formed in the embryonic primordium. It seems likely that the internal rudiments of the mesoderm and midgut separate from the ectoderm by delamination. The early elongation of the embryonic primordium is accompanied by a transverse demarcation into three equal parts, constituting a precociously delineated cephalic lobe, a pedipalpal segment and a posterior growth zone. At this stage, the germ band still lies at the postero-ventral pole of the yolk mass. The growth zone retain this position and proliferate further segments in a forward direction. As the length of the germ band increases, the segments which are already formed become more fully elaborated and the anterior end of the germ band pushes forwards until it reaches the anterior pole of the yolk mass.

During the formation of the four ambulatory segments of the prosoma, the anterior part of the cephalic lobe expands bilaterally to form a pair of precheliceral lobes and the posterior part is demarcated as a cheliceral segment. The stomodaeum invaginates between the procheliceral lobes. The development of prosomal limb buds does not begin until the first three opisthosomal segments have been formed by the

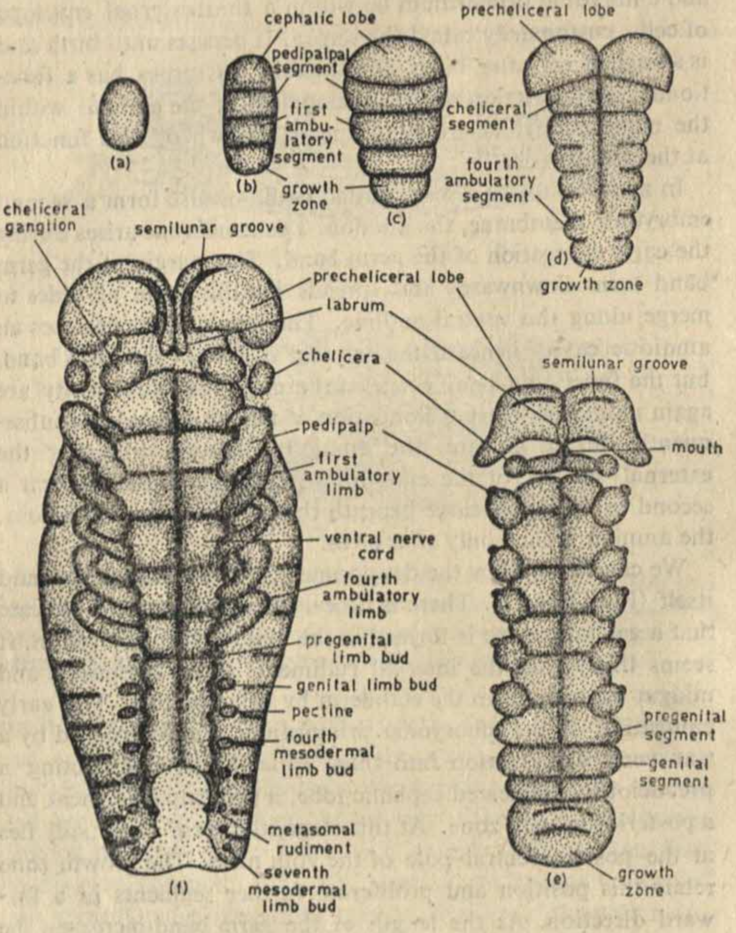

Fig. 10.6.11. Development of the germ band of *Buthus carpathicus*.
A—embryonic primodium.
B-D—formation of prosomal segments.
E—further development of the prosoma, formation of opisthosomal segments.
F—fully segmented germ band.

growth zone, but then takes place more or less simultaneously on all prosomal segments producing small cheliceral buds, large pedipalpal and first ambulatory limb buds and successively smaller pairs on the last three ambulatory segments. Accompanying the ventrolateral outpouching of the limb buds, the ventral parts of each segment rudiment thicken to form a pair of gangliòn rudiments on either side of midventral neural groove, but there is no bilateral separation of the germ band of formation of a ventral sulcus at any stage of development.

The proliferation of opisthosomal segments forwards along the ventral surface of the yolk mass continues until the seven mesosomal segments have been formed. By this stage, the enlarged procheliceral lobes have reached the anterior pole of the yolk mass. Seven pairs of 'mesosomal limb buds develop, accompanied by ventral ganglia and an intervening neural groove, as the last two mesosomal segments are formed.

After proliferating the seventh mesosomal segment flat against the yolk, the growth zone protrudes and proliferates the six metasomal segments as a tubular forwardly fixed, caudal papilla. The terminal unit formed by the growth zone is the telson, which subsequently develops as the sting. No limb buds are formed on the metasomal segments. Meanwhile the prosomal limbs grow longer and the mesosomal limb buds undergo various changes. The first or pregenital pair are soon resorbed. The second pair develop vestigially and merges to form the genital operculum. The third pair develop into the pectines. Behind the fourth to seventh pairs, invaginations initiate the formation of the four pairs of book-lungs and the limb buds gradually merge into the body surface as lung development continues.

Dorsal closure of the prosoma and mesosoma takes place by direct upgrowth of the lateral margins of the segments towards the dorsal midline, passing over the surface of the yolk mass beneath the external serosa, and carrying the edge of the amnion with them. At the anterior end, a pair of long, narrow, lateral lobes develop at the median edges of the precheliceral lobes. The swollen posterior ends of the lateral rudiments merge and give rise to the labrum, overhanging the mouth. Simultaneously, a pair of semilunar grooves develops around the anterolateral margins of the precheliceral lobes. The outer margins of the semilunar grooves then overgrow the broad surfaces of the precheliceral lobes in a posteromedian direction and merge into the labrum. The chelicerae move forwards to a preanal position on either side of the labrum, while the pedipalps become para-oral, with medially directed gnathobases. In spite of the peculiarities of development in the embryos of ovoviviparous scorpions, development of the anterior end retains the features common to all arachnids.

Gastrulation is by the release of vitellophages from the germ disc which spread around the surface of the yolk mass. A compact group of cells said to be proliferated inward from the centre of the germ disc then forms the primordial germ cells. Finally, in succession, two layers of cells appear to be individuated within the inner part of the germ disc. The innermost layer is the midgut rudiment, and the layer next to the columnar ectoderm is the mesoderm rudiment.

The vitellophages derived from the germ disc spread around the surface of the yolk mass to some extent, but do not invade the yolk mass or take part in the formation of the definitive midgut epithelium. The latter is formed by proliferation and spread of the midgut rudiment around the yolk mass, accompanying the growth and segmentation of the germ band. As the forwardly flexed metasoma develops, a tubular extension of the midgut epithelium penetrate into the rudiment. The short proctodaeum arises late in development, as an invagination in the telson. The stomodaeum forms earlier, in the usual way. During dorsal closure, the upgrowing mesoderm cuts into the midgut sac, defining the digestive diverticula. Some yolk is still present in the midgut at birth.

The mesoderm in each segment rudiment of the germ band separates in the usual way, first longitudinally, then transversely, into paired somite rudiments. The six prosomal and seven mesosomal pairs become hollow and trilobed, but the six metasomal pairs lack appendicular lobes. During dorsal upgrowth, the dorsolateral lobes of the somites give off blood cells. The dorsal edges of the adjacent somatic mesoderm gives off cells which differentiate as dorsal longitudinal muscles, before separating from the ectoderm and merging with the underlying splanchnic mesoderm to form the pericardial floor. Narrow pericardial coelomic tubes persist in the pericardial floor. The principal product of the splanchnic mesoderm is splanchnic muscle, and of the appendicular lobes, somatic muscle but the details of these aspects of development are not clear.

The coxal glands are paired. The duct rudiments develop as the usual funnel-like outgrowths of the posteroventral walls of the four pairs of coelomic sacs in the ambulatory segments. Those of the first, second and fourth segment are transient vestiges, but those of the third ambulatory segment develop as coiled ducts with coelomic end sacs. Typical, short, ectodermal exit ducts arise at the bases of the third pair of ambulatory limbs. The gonads are formed mainly from the coelomic sacs of the genital segment, which unite and receive contribution from adjacent somites in producing the genital coelom. The gonoducts are coelomoducts which grow out from the somatic walls of the genital coelomic sacs, curve down on either side of the gut and meet a midventral ectodermal invagination at the genital pore. The germ cells remain grouped in the mesoderm and the growth zone turns forwards and forms the caudal papilla. After a temporary sojourn in the last mesodermal segment, the germ cells migrate forwards and enter the genital coelom.

The development of the embryonic ectoderm develops, as embryonic primordium separates from the remainder of the blastoderm, which overgrows the primordium to form the serosa. All of the embryonic ectoderm arises from the embryonic primordium and the germ band remains narrow throughout its length. The bilateral halves of the embryonic ectoderm are conjoined midventrally from the moment of their formation. The embryonic ectoderm of the embryonic primordium includes the pedipalpal ectoderm rudiments as well as the cephalic lobe ectoderm anteriorly and the growth zone ectoderm posteriorly. Thus the growth zone does not proliferate pedipalpal ectoderm, though it buds off the ectoderm of the remaining segments as a succession of short blocks along the ventral surface of the yolk mass. The last six rudiments, making up the metasoma, are not concentrically compressed onto the posterior face of the yolk mass, but are carried beyond the yolk as a tubular, forwardly flexed, caudal papilla. Finally the peripheral ectoderm of the embryonic primordium has a specialized development as an amnion.

The ventrolateral embryonic ectoderm, median to the limb buds in the segments which develop limb-buds and in the corresponding position in limbless segments, thickens bilaterally to form a pair of segmental ganglion rudiments (Fig.10.6.12). Simultaneously, a pair of more complex ganglia develops from the

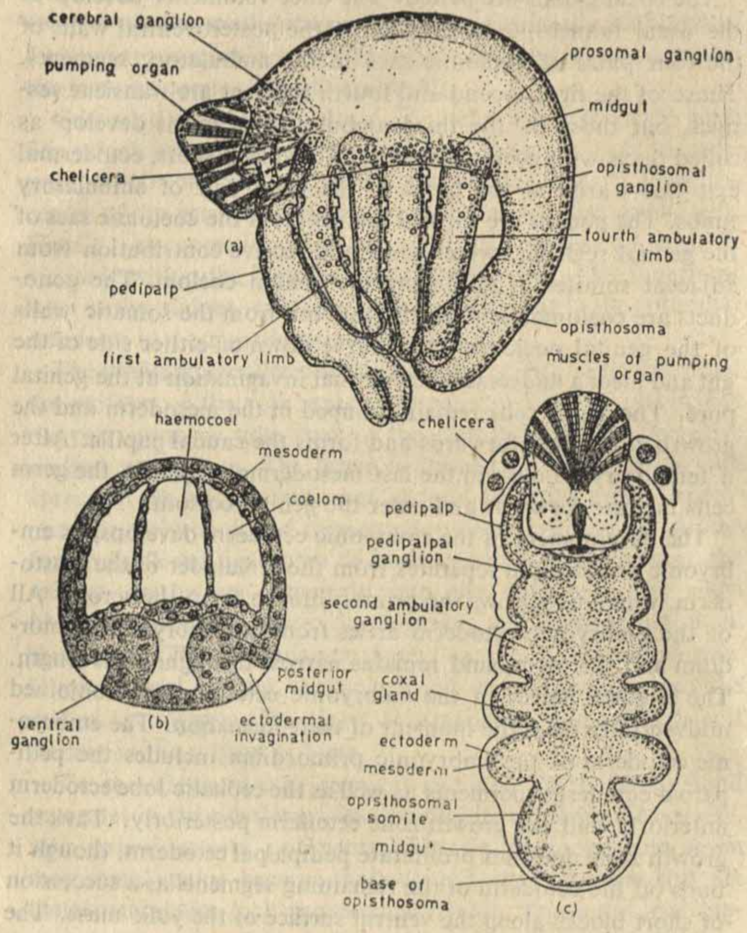

Fig. 10.6.12. Development of prosomal ventral ganglia in *Neobisium*.
A—lateral view of embryo with well developed opisthosoma and developing ventral ganglia.
B—T.S. through middle region of opisthosoma of the same embryo.
C—frontal section through embryo at the level of the prosomal ganglia.

ectoderm of the precheliceral lobes (Fig. 10.6.13). In later development, the ganglia of the cheliceral segment move forwards on either side of the mouth and unite with the precheliceral ganglia to complete the supra-oesophageal ganglion, but retains a post-

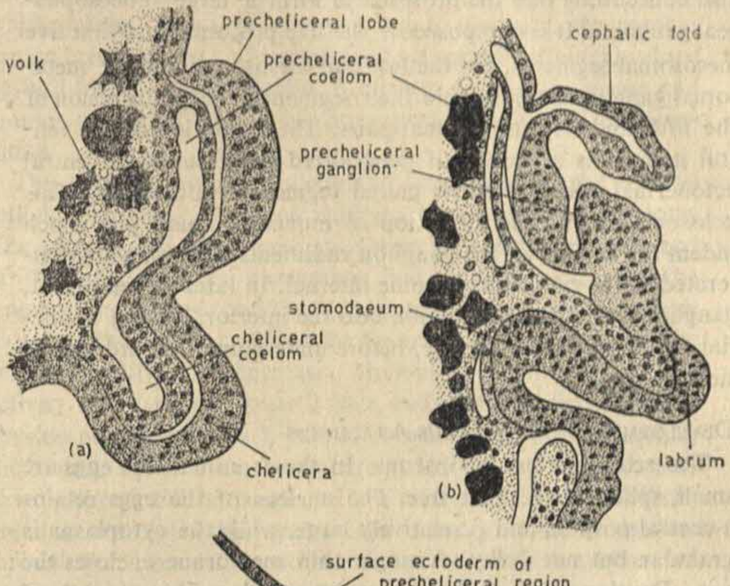

Fig. 10.6.13. Development of the precheliceral ganglia of *Heptathela*.

A—Parasagittal section through precheliceral lobe and chelicera before the onset of formation of semilunar grooves.

B—S.S. through anterior end after onset of overgrowth of semilunar grooves.

C—S.S. through anterior end during closure of semilunar groove.

anal commissure. Most or all of the remaining ventral ganglion also concentrate into the prosoma to form a large suboesophageal ganglion. It is composed of the five prosomal and first five mesosomal segments, but the last two mesosomal and six metasomal ganglia remain within their segments, except for fusion of the fifth and sixth metasomal pairs. The formation of the ventral ganglia is a process of generalized proliferation of ventral ectodermal cells to produce paired segmental thickenings. This is associated with the formation of numerous small pits which indent the surface of the ganglion rudiments. The pits are obliterated as the rudiments become internal. In later development, ganglionic thickenings separate into the interior, leaving epithelial ectoderm at the surface, before undergoing forward migration and fusion.

DEVELOPMENT IN VIVIPAROUS ARACHNIDS

The scorpions are viviparous. In these animals the eggs are small, spherical and yolk free. The nucleus of the eggs retains a central position and is relatively large, while the cytoplasm is granular but not yolky. A single thin membrane encloses the egg. Development of the embryo proceeds within the lateral ovarian follicle in which the egg is formed. They display a total cleavage. Cleavage is preceded by the release of two polar bodies which mark the anterior end of egg. The cleavage division then proceeds as regular, total equal divisions, passing through 2,4,8,16 and 28 cell stage before becoming more irregular. The result is spherical mass of small cells surrounding a small central blastocoel. When the egg first begins to cleave, it is surrounded by a membrane and lies within a lateral ovarian follicle. As cleavage continues the follicle wall shrinks away from the egg, leaving a space, except anteriorly, where the cleavage morula remains attached to the distal follicle cells. The later proliferate as an enveloping layer around the embryo, the embryonic capsule. At the same time, other distal follicle cells produce a terminal plug of large clear cells. These cells subsequently develop into a feeding structure called the appendix, through which nutriment is passed from the maternal tissue to the growing embryo.

Before the embryo begins to elongate, the embryonal capsule breaks down and is replaced by a cellular envelope developed by proliferation of the polar body cells which attach the embryo to the follicle wall. This cellular envelope is called as the trophamnion. When the embryo begin to elongate, the trophamnion breaks down and is replaced by a second cellular envelope derived from the follicle cells. According to this, the serosa and amnion of buthid embryo are not represented in yolkless scorpions.

The embryo elongates along the long axis of the ovarian follicle, the cavity of which enlarges as the embryo develops. The anterior end of the embryo is directed towards the free end of the follicle. When elongation first begins, increasing the length of the embryo to 200μ, a large mouth develops by invagination of the anterior wall of the embryo. As the length of the embryo continues to increase, obviously due mainly to the activity of a posterior growth zone, rudiments of the chelicerae develop precociously as a pair of forward protrusions flanking the mouth. By the time the embryo is 750 μ long, the chelicerae have gripped the appendix at the end of the ovarian follicle, pressing the appendix against the mouth channels leading through the appendix provide an access for nutrients synthesized in the maternal digestive glands.

During the first elongation of the embryo to 750 μ the body becomes subdivided into an anterior prosomal rudiment, a median mesosomal rudiment with three pairs of dorsolateral protrusion, and a growth zone. This subdivision gives the first indication of the second important specialization of the mesosoma. As the length now increases to 2 mm, the prosoma remains short and devoid of pedipalpal and ambulatory limb rudiments, but the mesosoma acquires the full complement of seven segments, each with a pair of dorsolateral diverticula (Fig. 10.6.14). The midgut does not extend into these protrusions but the protrusions themselves persist throughout development, presumably as exchange surface between the embryo and maternal tissue.

The embryo continues to grow longer as the metasomal segments are added one by one and the large mesosoma takes on a dorsoventral flexure with the dorsal surface convex and the dorsolateral diverticula protruding from it. Small pedipalpal and ambulator limb buds develop on the prosoma and a small labral lobe pushes out between the chelicerae and the anterior

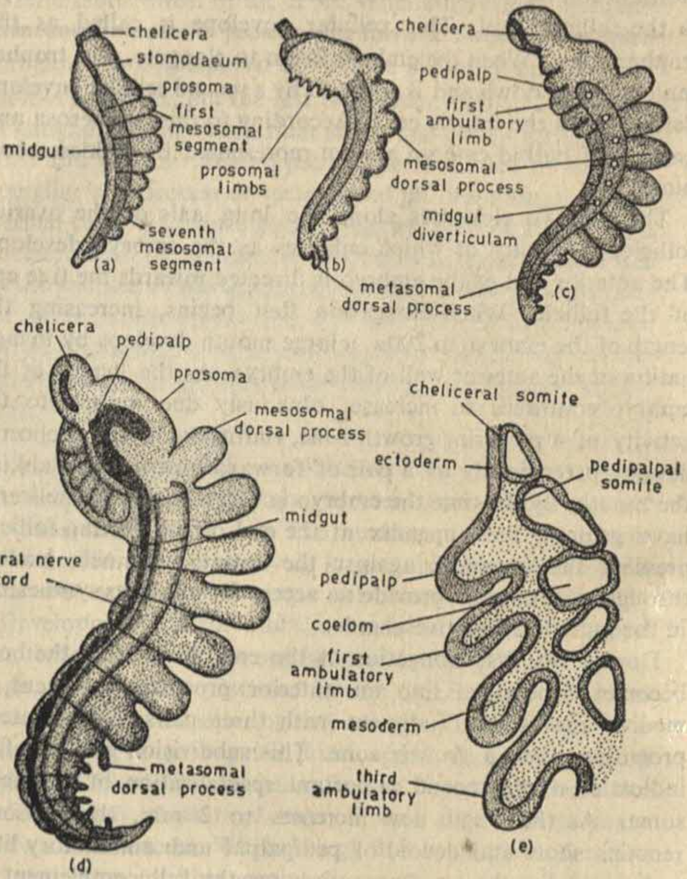

Fig. 10.6.14. A-D—External development of the embryo of *Heterometrus*.
A—3 mm embryo.
B—4 mm embryo.
C—6 mm embryo.
D—9 mm embryo.
E—L.S. through prosomal limb buds and associated coelomic sacs of a 4 mm embryo.

end of the body. The metasomal segments develop dorsolateral diverticula smaller than these on the mesosoma. By the time the embryo is 6-6.5 mm long, the dorsoventral flexure of the large mesosoma is increased. The metasoma shows a reciprocal upward flexure. The prosomal limb buds are now longer, and the mesosoma has developed limb buds on the second to sixth segments. Those of the second and third segments are the rudiments of the genital operculum and pectines. The limb buds on the third to sixth segments are resorbed while book-lungs invaginate on these and the seventh mesosomal segment in the usual way. No limb vestiges are developed on the pregenital or seventh mesosomal segment. The full external appearance of the scorpion is attained during the later growth of the embryo to its maximum length of 19 mm. The dorsolateral protrusion gradually retract but are not finally resorbed until after birth. Simultaneously, the chelicerae retain their specialization until after birth acting as a part of the feeding apparatus of the embryo throughout development.

Gastrulation in the viviparous scorpions is little known. In the genus, *Heterometrus* (Fig. 10.6.15), the small, spherical blastula enlarges and exhibits a unipolar ingression of cells which fills the central cavity since the inner mass subsequently segregated into mesoderm and midgut rudiments, the region of ingression is presumably the equivalent of the germ disc of ovoviviparous species.

The mode of development of midgut in scorpions is unique among arachnids when the small gastrula begins to increase in length, the internal mass of cells withdraws from the anterior end leaving a space. The anterior part of the external wall then invaginates into this space, forming a large stomodaeum with the mouth directed towards the axial canal of the appendix. With further enlargement of the embryo, some of the internal cells accumulate on the outer surface of the stomodaeum and differentiate as the stomodaeal muscle. An opening forms at the free end of the stomodaeum, through which nutritive material from the appendix enters the embryo. The internal cells immediately around the space receiving this fluid unite to form a midgut sac attached to the end of the stomodaeum. The remainder of the internal cells form mesoderm. As the embryo grows longer the midgut sac extends with it and become tubular. The stomodaeum has suctorial action, pumping nutrient fluid from the appendix into the midgut. In later development, the midgut develops six pairs of lateral diverticula. The diverticula become branched, the first pair forming an anterior pair

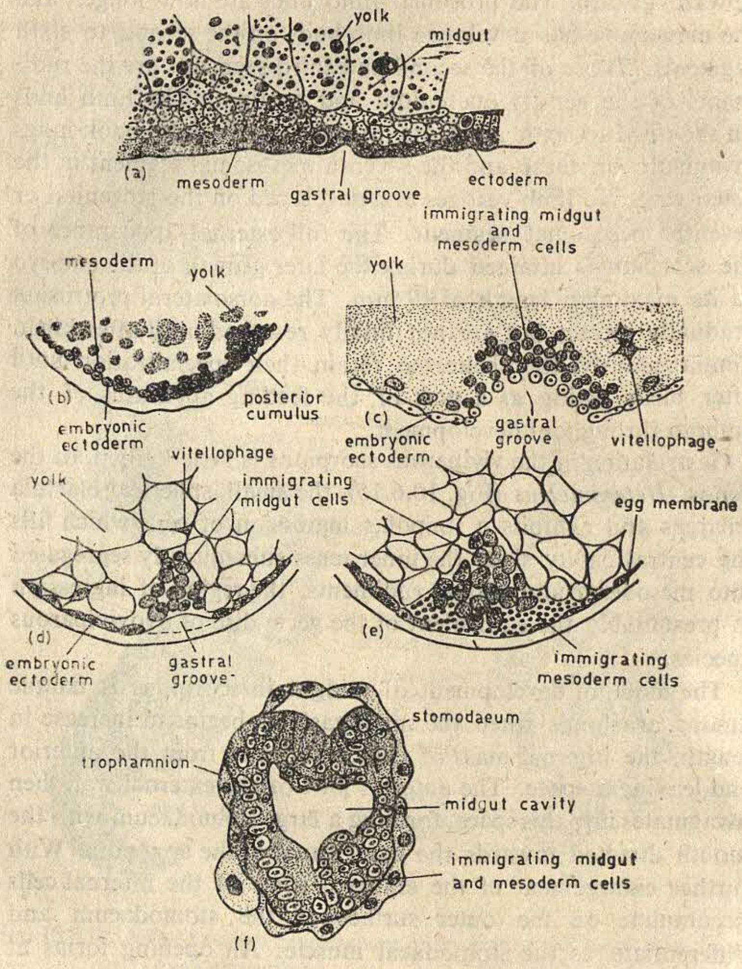

Fig. 10.6.15. Gastrulation in chelicerate embryos.

A—T.S. through early gastral groove of *Tachypleus*.

B—S.S. through the embryonic primordium of *Latrodictus*, following onset of gastrulation.

C—T.S. through the gastral groove of *Ischnocolus*.

D-E—T.S. through the gastral groove of *Ornithodorus* showing median vitellophage proliferation and bilateral mesoderm proliferation.

F—Frontal section through gastrulating embryo of *Heterometrus*.

of digestive glands opening into the stomach, the last five pairs merging to form a pair of posterior digestive glands with five pairs of ducts opening into the midgut. Feeding continues until embryonic development is completed and the young are born. The short proctodaeum invaginates late in the development but does not become connected with the midgut until after birth. In all respects, the development of the gut in yolkless scorpion embryo proceeds in the same manner as in yolk-filled embryos, except that the midgut epithelium develops precociously around a central mass of nutrient fluid rather than around a yolk mass. Malpighian tubules in both types of embryos arise as evaginations of the midgut wall at the junction of the mesosoma and metasoma, behind the last pair of digestive diverticula.

Mesodermal development in yolkless scorpion embryos shows a number of specializations. Paired mesodermal bands develop ventrolaterally on either side of the gut, but do not subdivide directly into paired somites. The cheliceral pair of somites individuates precociously at the anterior ends of the mesodermal bands and develop as cheliceral and labral musculature, but the next emphasis is on development of the mesoderm of the enlarged mesosoma. Here, the mesodermal bands give off numerous blood cells and then instead of forming paired somites, disperse to form a series of trabeculae connecting the body wall to the midgut wall. The functional significance of the trabeculae is not known. In the later development of the mesosoma, the trabeculae differentiate as somatic and splanchnic musculature and dorsally as the wall of the heart. When the limb buds of the prosoma develop, the underlying mesodermal bands segment to form the corresponding hollow somites with limb bud diverticula. Similarly, at the anterior end of the mesosoma, the genital and pectinal segments also develop paired, ventrolateral somites before they develop limb buds. A small transitory pair of pregenital somites accompanies them as a vestige of an almost suppressed pregenital segment.

The development of coxal glands, gonads, gonoducts and the ectoderm in scorpions follows the same pattern as that of buthids. The basic development of the nervous system is like that of *Buthus*. But the formation of the ventral ganglion is preceded by a segregation of large neuroblasts from an external layer of epithelial cells in the ventral ectoderm of the embryo. The neuroblasts then proliferate the ganglion cells. The median eyes arise as ectodermal invaginations. The optic ganglia usually develop from the deepest parts of the eye invaginations.

The scorpions develop four pairs of book lungs on the fourth to seventh opisthosomal segments. The limbs of the third opisthosomal segment develop as pectines, with no indication of transient book-lung formation. Each book-lung originates as an ectodermal invagination behind an opisthosomal limb bud (Fig. 10.6.16). The invagination increases in size, forming a pulmonary sac, while the limb bud merges back into the general ectoderm of the segment. The anterior wall of the pulmonary sac, which is a direct continuation of the posterior wall of the limb bud, develops projecting lamellae.

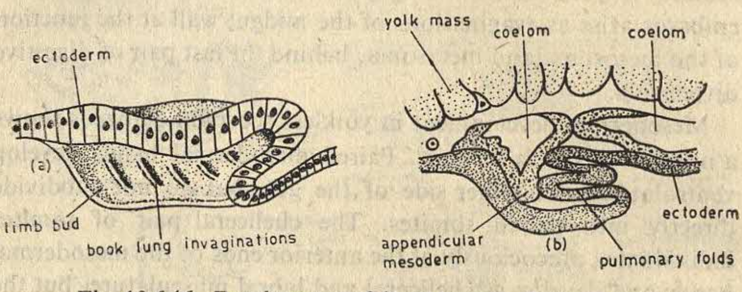

Fig. 10.6.16. Development of book-lungs in Spider, *Attus*.
A—first abdominal appendage at the onset of formation of the folds of the book-lung, posterior view.
B—S.S. of the same appendage at a later stage in infolding of the book-lung.

DEVELOPMENT IN XIPHOSURA

The egg of *Limulus polyphemus* is ovoid, with an average diameter of 2 mm while that of *Tachypleus* average 2.5 mm. In spite of having relatively large eggs, the xiphosura still retain a cleavage pattern more primitive than that of any arachnid. The first few nuclear divisions are intralecithal, but that surface furrowing then begins to develop at the pole adjacent to the dividing nuclei, indicative of the onset of total cleavage. Spreading from this pole, the egg becomes first divided into numerous large, equal, yolky cells with central nuclei. Divisions then become concentrated in the peripheral cells, which gives rise to a columnar blastoderm. The central cells remain undivided filling the space within the forming blastoderm. The central cells give rise to the midgut epithelium. The blastoderm secretes a blastoderm cuticle. The chorion breaks down some days after the blastodermal cuticle has been formed and the cuticle persists as a protective membrane until hatching.

The most generalized pattern of external development is found in the xiphosura. The first external indication of development beyond the blastoderm stage in *Limulus* is the formation of a small ventral area of concentrated blastoderm cells, quickly followed by the formation of a second similar area immediately behind the first. The two areas soon merge by thickening of the intervening region, to form an oval embryonic primordium which is broader posteriorly than anteriorly. While merging is taking place a median gastral groove arises anteriorly and extends along the midline of the embryonic primordium. The remainder of the blastoderm attenuates to form a dorsal extra-embryonic ectoderm over the large mass of yolky cells.

The embryonic primordium now enters into elongation and segmentation as a germ band (Fig. 10.6.17). A transverse line, situated behind the anterior end of the gastral groove, demarcates a small cephalic lobe from a larger growth zone. Behind this line, the pedipalpal and four ambulatory segments of the prosoma are successively delineated as the growth zone augments the length of the germ band. The gastral groove progressively closes anteriorly and extends posteriorly along the midline of the forming segments but remains open at the extreme anterior end as the stomodaeal invagination. The prosomal segments broaden as they are formed, so that the prosomal part of the xiphosuran germ band takes up most of the ventral surface of the egg when the opisthosomal segments begins to be proliferated by the growth zone. The segment rudiments are situated at the curved posterior pole of the egg. Furthermore, each segment rudiment is short and broad when first formed. The rudiments are therefore laid down as a concentric series on the posterior pole of the yolk mass. The gastral groove continues to extend in a posterior direction along the midline during formation of the opisthosomal segments, but is then obliterated in an anteroposterior wave. While the rudiments of the opisthosomal segments are being formed, the prosomal segments develop paired, ventrolateral limb buds on the pedipalpal and

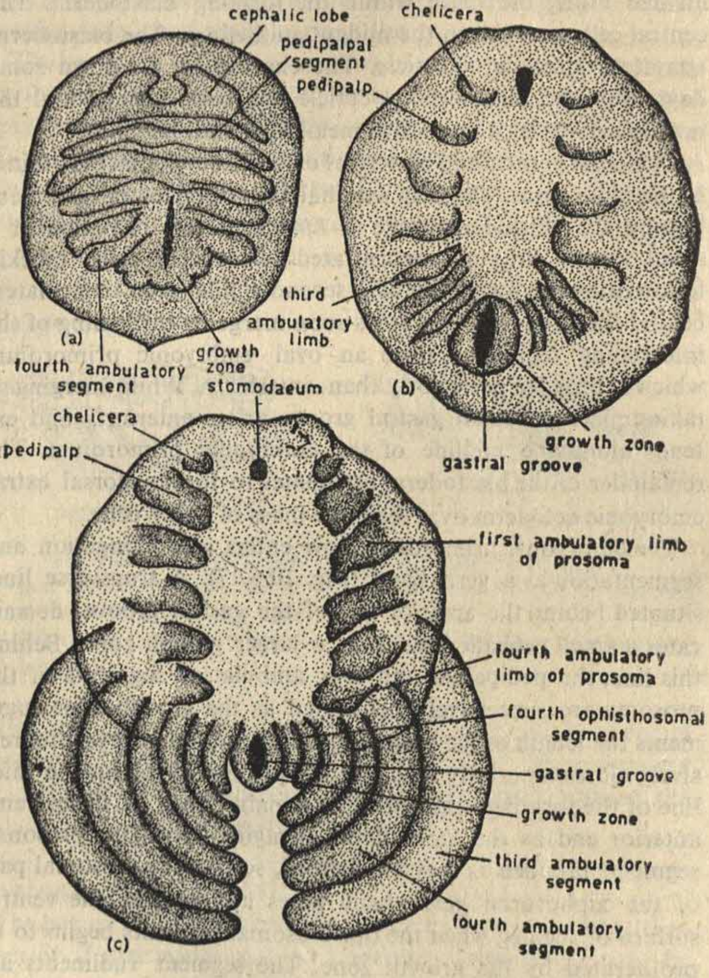

Fig. 10.6.17. The segmenting germ band of *Tachypleus*.
A—during formation of the prosomal segment rudiments.
B—at the onset of formation of the opisthosomal segment rudiments.
C—late in formation of the opisthosomal segment rudiments.

four ambulatory segments. A pair of cheliceral limb buds also arise posterolaterally on the cephalic lobe, behind the level of the stomodaeum. No trace of precheliceral limb buds has ever been observed.

In the subsequent development of the opisthosomal segments, each segment rudiment increases in length anteroposteriorly (Fig. 10.6.18) and also undergoes a dorsal closure, so that the concentric arrangement of the early segment rudiments is supplemented by an antero-posterior succession of gradually diminish-

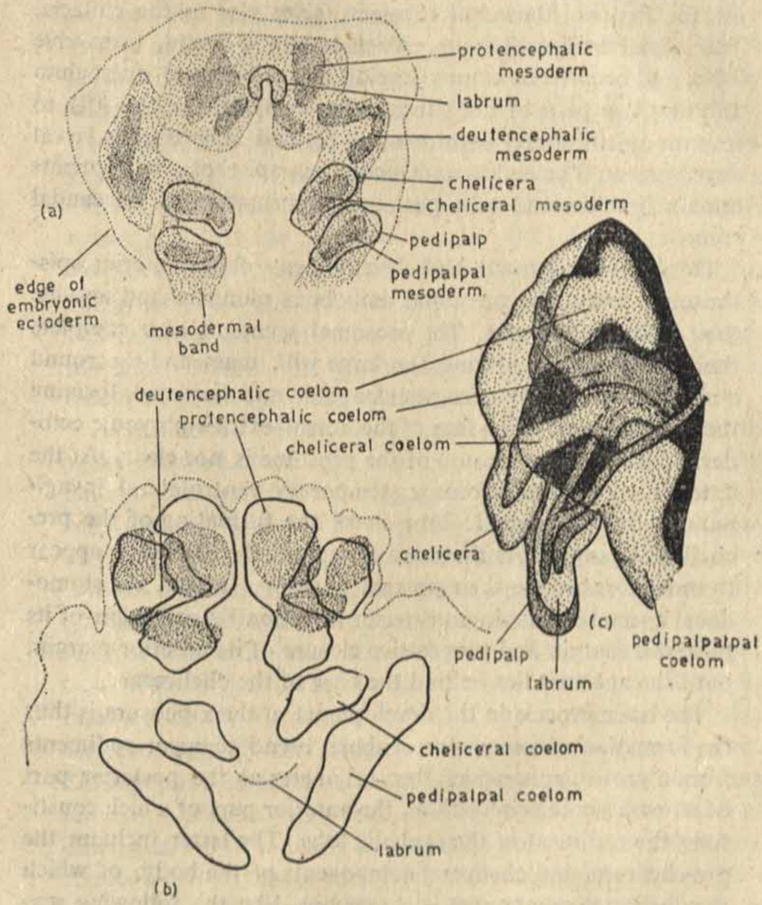

Fig. 10.6.18. *Pardosa hortensis.*

A—Distribution of mesoderm and aggregation of somite rudiments beneath the anterior end of the segmenting germ band.

B—Distribution of anterior somites during inversion.

C—Diagrammatic reconstruction of anterior end of embryo after inversion.

ing tubular segment. The large mass of yolky cells remains mainly within the prosoma. Limb buds also develop on the opisthosoma during this phase of development. The first pair, on the first opisthosomal segment, gives rise to the chilaria. The second to fourth pairs, which arises as broad, transverse lobes and become biramous develop into the genital operculum and first two pairs of book lungs. The book gills of the fifth to seventh opisthosomal segments are formed later during larval development. The eighth and succeeding opisthosomal segments remain limbless and take part in the formation of the caudal spine.

During the outgrowth and development of the anterior opisthosomal limbs, the prosomal limb buds elongate and assume their definitive features. The prosomal segments also complete their dorsal closure around the large yolk mass and the round embryo subsequently becomes elongated and flattened, forming the trilobite larva. The fate of the dorsal extra-embryonic ectoderm during dorsal closure of the prosome is not clear. At the anterior end of the prosoma, temporary ventrolateral invaginations on the cephalic lobe mark the formation of the precheliceral ganglia. As these are closed off, the chelicerae appear to move forwards to their preanal position. In fact, the stomodaeal aperture shifts in a posterior direction by extension of its posterior margin and progressive closure of its anterior margin, until the aperture lies behind the base of the chelicerae.

The basic process in the development of the xiphosura is thus the formation of a sequence of short, broad segment rudiments from a growth zone which first originates as the posterior part of an embryonic primordium, the anterior part of which constitutes the rudiment of the cephalic lobe. The latter includes the pre-cheliceral and cheliceral components of the body, of which the cheliceral component is a segment, like the following segments, but the precheliceral component is a region whose morphological composition is more complex. A midventral gastral groove extends temporarily through all segments. The relatively large prosoma develops by dorsal closure around the large yolk mass. The smaller, conical opithosoma develops mainly behind the yolk mass.

The gastrulation in xiphosura is as follows. The mass of large, polygonal, yolky cells which lies within the blastoderm is the rudiment of the midgut. The presumptive midgut thus come to occupy its internal position as a result of total cleavage and subsequent delamination of the blastoderm, and exhibits no gastrulation movement. The mesoderm, on the other hand, is proliferated into the anterior along the length of the gastral groove, spreading outward between the superficial ectoderm of the germ band and the large, internal midgut rudiment. It can be inferred from this that the presumptive mesoderm occupies the ventral line of the embryonic primordium and retains a slight gastrulation movement, in the form of a shallow insinking, before entering into proliferative organogeny. At the anterior end of the gastral groove, the invaginated cells from the wall of the stomodaeum and the external aperture of this part of the groove persists to form the mouth when the remainder of the groove undergoes antero-posterior closure. The proctodaeum develops very late as an independent invagination on the telson.

As mentioned before, the midgut develops wholly from the yolky cells which fill the interior of the blastoderm. As the embryo changes shape during later development, the mass of cells become, first ovoid, then hemispherical in the prosoma and cylindrical in the opisthosoma when the mesoderm extends towards the dorsal midline of the prosoma, it cuts into the midgut mass, subdividing the lateral portions of the latter into six pairs of segmental lobes, the rudiments of the digestive diverticula. During the further elaborations of the musculature, the first, second, fifth and sixth pairs of lobes, which retain their connections with the midgut as two pairs of hepatic ducts. Throughout this series of shape changes, the midgut rudiment remain as mass of yolky cells, yolk resorption, accompanied by differentiation of the cells as epithelial cells of the midgut and digestive diverticula, is completed only after hatching has taken place. The stomodaeum flexed backwards as it grows into the interior and comes into contact with the anterior face of the midgut. The stomodaeal wall differentiates as the epithelium of the buccal cavity, oesophagus and proventriculus and secretes a cuticular lining. The proctodaeum forms only a short, posterior terminations of the gut.

The mesoderm enters along the ventral midline of each successive segment of the germ band and spreads laterally beneath the ectoderm of the segment as a pair of transverse somite rudiments. These then split internally forming paired hollow somites. The prosomal pairs develop well in advance of the opisthosomal pairs, which are successively serially delayed. The somites have thick somatic and thin splanchnic walls (Fig. 10.6.19). In the segments in which limb buds are developed, each somite develops an appendicular lobe which penetrates into the corresponding limb buds. The somites extend upwards towards the dorsal midline during later development and the dorsal margins of all somites behind the second ambulatory segment participate in the formation of the heart. The paired lateral ostia of the heart develop at the intersegmented junctions between successive somite pairs. Commensurate with the pattern of dorsal closure, the development of the heart begins at the posterior end of the opisthosoma and proceeds in the anterior direction. On either side of the heart, the somatic walls of the somites separate from the overlying ectoderm and merge with the splanchnic walls to form the pericardial floor with its residual pericardial coelomic cavities. The appendicular lobes and adjacent somatic mesoderm give rise to somatic musculature, while the major part of the splanchnic wall of each somite presumably develops as splanchnic musculature.

The coxal glands of *Limulus* originate from paired, hollow rudiments derived from the ventral portions of the somites of the six prosomal segments. The cheliceral and fourth ambulatory rudiments are transient, but the remaining pairs become linked together longitudinally to form the lobes of the two coxal glands. The pair of the third ambulatory segment also develop looped, tubular extensions which form the ducts of the glands, opening to the exterior through short ectodermal ducts at the bases of the third ambulatory limbs.

The development of the xiphosuran reproductive system is similar to that of the scorpions.

The general course of development of the ectoderm as epidermis is as follows. The blastoderm cells outside the area of the embryonic primoridum participate in the initial development of embryonic ectoderm and that only a portion of them pass through a phase of temporary attenuation as dorsal yolk sac ectoderm. The ventral embryonic primordium elongates due to proliferation by posterior growth zone and that the anterior

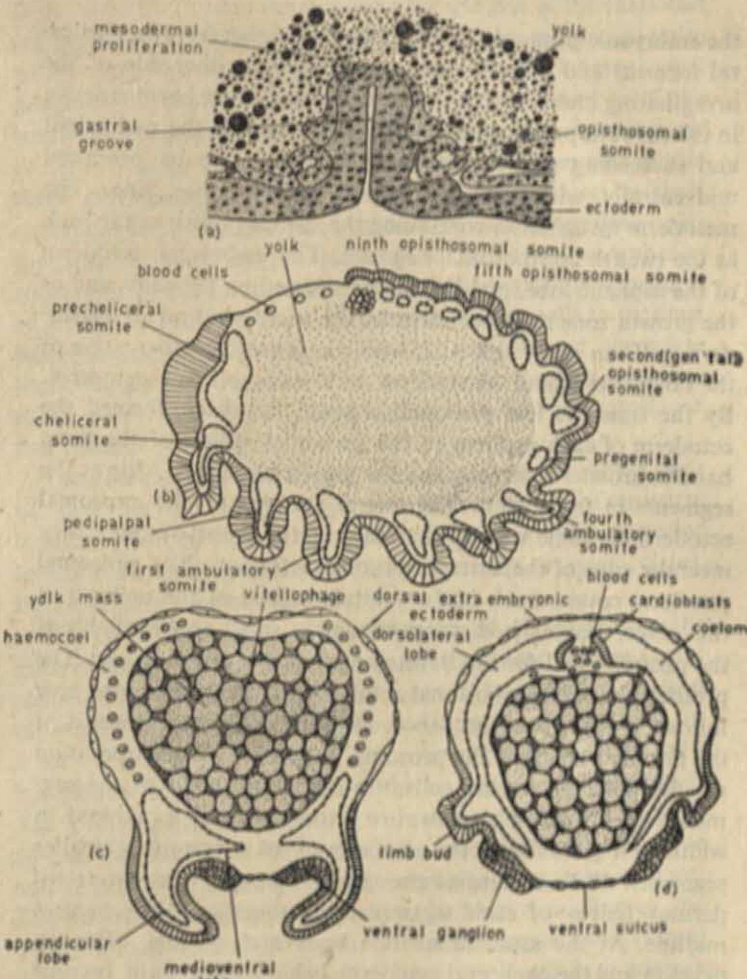

Fig. 10.6.19. A—T.S. through the gastral groove during the proliferation of opisthosomal somites in *Tachypleus*.
B—Parasagittal section through the segmented germ band of *Agelena*.
C-D—T.S. showing the further development of the prosomal (c) and opisthosomal (d) somites of *Thoridium* as inversion begins.

part of the primordium in front of growth zone, constitute the rudiment of the cephalic lobe only. The embryonic ectoderm of the embryonic primordium i.e. of the pre-cheliceral lobe, cheliceral segment and growth zone, is bilateral on either side of the invaginating mesoderm. Now, as the growth zone become active in the forward proliferation of the rudiments of the pedipalpal and succeeding segment, mesoderm continues to be produced midventrally, with embryonic ectoderm on either side. The mesoderm invaginates, continuing the gastral groove as far back as the twelfth opisthosomal segment. The embryonic ectoderm of the cephalic lobe, pedipalpal and succeeding segments and of the growth zone itself broadens by the incorporation of blastoderm cells on either side of it with compensatory attenuation of the dorsal to lateral blastoderm as dorsal yolk sac ectoderm. By the time the last prosomal segment has been formed the ectoderm of each segment of the prosomal region of the germ band is broader than long and the growth zone is as wide as the segments in front of it. The lateral margins of the prosomal ectoderm lie some way up the sides of the embryo where they meet the edge of the extra-embryonic ectoderm. The prosomal ectoderm covers much of the ventral surface of the embryo at this stage, leaving a disproportionately small area available at the posterior end for the development of the opisthosoma. The proliferation of opisthosomal ectoderm by the growth zone now follows a diminishing sequence. Obviously this is the reverse of the growth pattern of the prosoma. Instead of the incorporation of additional blastoderm cells into the lateral parts of the segment rudiments, the growth zone ectoderm itself is reduced in width as it gives rise to the ectoderm of the successively smaller segments. With closure of the gastral groove, the two ectodermal halves of each segment come together in the ventral midline. At the same time, limb buds and ganglia begin to develop and the cheliceral ectoderm behind the mouth become delineated from the precheliceral ectoderm in front of and around the mouth.

The basic development of the nervous system is alike throughout the chelicerates. The ventrolateral embryonic ectoderm, median to the limb buds in the segments which develop limb buds, and in the corresponding position in limbless segments, thickens bilaterally to form a pair of segmental ganglion rudiments (Fig. 10.6.12). Simultaneously, a pair of more complex ganglia develop from the ectoderm of the precheliceral lobes

(Fig. 10.6.13). In later development the ganglia of the cheliceral segment move forwards on either side of the mouth and unite with the precheliceral ganglia to complete the supra-oesophageal ganglion, but retains a post-oral commissure. Most or all of the remaining ventral ganglia also concentrate into the prosoma to form a large suboesophageal ganglion. The ganglia of the three gill-bearing segments remain separate and retain their segmental location, but those of the sixth and succeeding opisthosomal segments merge together to form a terminal opisthosomal ganglion. The formation of ventral ganglion is a process of generalized proliferation of ventral ectoderm cells to produce paired segmental thickenings. In lateral development, the ganglionic thickenings separate into the interior, leaving epithelial ectoderm at the surface, before undergoing forward migration and fusion.

The development of the book-gills arise from the limb buds of the third to seventh opisthosomal segments. Transverse lamellae arises as outpouchings of the ectoderm of the posterior face of each limb bud, the first lamella being the most distal, the remainder progressively more proximal.

The xiphosuran presumptive midgut, which comprises the mass of large yolk cells enclosed by the blastoderm during total cleavage, has no place in the blastoderm and no gastrulation movement. The presumptive stomodaeum is immediately in front of the anterior end of the presumptive mesoderm and invaginates as the anterior end of the wall of the gastral groove. The presumptive proctodaeum comprises a posterior area of cells in the ventral midline behind the ectodermal growth zone and makes a late, independent invagination into the interior. The presumptive mesoderm occupies the ventral midline of the embryonic primordium, extending from the presumptive stomodaeum to the presumptive growth zone ectoderm as a narrow band of cells. At gastrulation, these cells invaginate, forming a gastral groove with the invagination stomodaeum at its anterior end. The presumptive mesoderm remains superficial in the growth zone and continues the process of gastral groove formation progressively along the growing germ band.

The presumptive ectodermal areas at first occupy only a relatively small part of the ventral surface of the blastoderm but are gradually augmented by the incorporation of many of the lateral and anterior blastoderm cells, with simultaneous attenuation and spread of the dorsal part of the blastoderm as extra embryonic ectoderm. The presumptive ectoderm comprises a presumptive head lobe area around the presumptive stomodaeum and anterior end of the mesoderm, a bilateral area of presumptive pedipalpal ectoderm on either side of the mesoderm and an area of presumptive growth zone ectoderm around the posterior end of the mesoderm.

DEVELOPMENT OF TICKS AND MITES

The ticks retain quite large eggs, ovoid in form and centrolecithal. The nucleus lies in the centre, surrounded by typical cytoplasmic halo, and the cytoplasm is filled with yolk spheres of various sizes, held in a cytoplasmic reticulum which unites with a thin, transparent vitelline membrane and thicker, two layer chorion surround the egg. Both these membranes are surrounded by the egg surface. The mites eggs are generally smaller, nucleus central, together with evenly distributed yolk in the cytoplasm, but the presence or absence of a periplasm is not clearly established. Both chorion and a vitelline membrane are present. Intralecithal cleavage leading directly to blastoderm formation without the intervention of pyramid formation is a feature of the development in Acarina. A eight synchronous cleavage division precede the arrival of 256 nuclei at the egg surface in the ticks and the formation of a uniform, low cuboidal blastoderm follows. In mites, the ovoid egg first divides transversely and equally. Each daughter cell then divides along the anteroposterior axis of the egg, but the planes of division of the two cells are perpendicular to one another. A similar division in the alternate plane separate eight equal blastomeres with more or less peripheral nuclei. Further division proceed with increasing asynchrony, first cutting the eight blastomeres into smaller cells with peripheral nuclei, then cutting off a blastodermal layer of yolk free cells from internal, anucleate yolk masses which fuse together once more. The final result, a uniform low, cuboidal blastoderm around a unitary yolk mass.

The external features of development in ticks and mites follows similar course (Fig. 10.6.20. and 10.6.23). The first external sign of development is the formation of a small, midventral germ disc with a temporary gastral groove at its

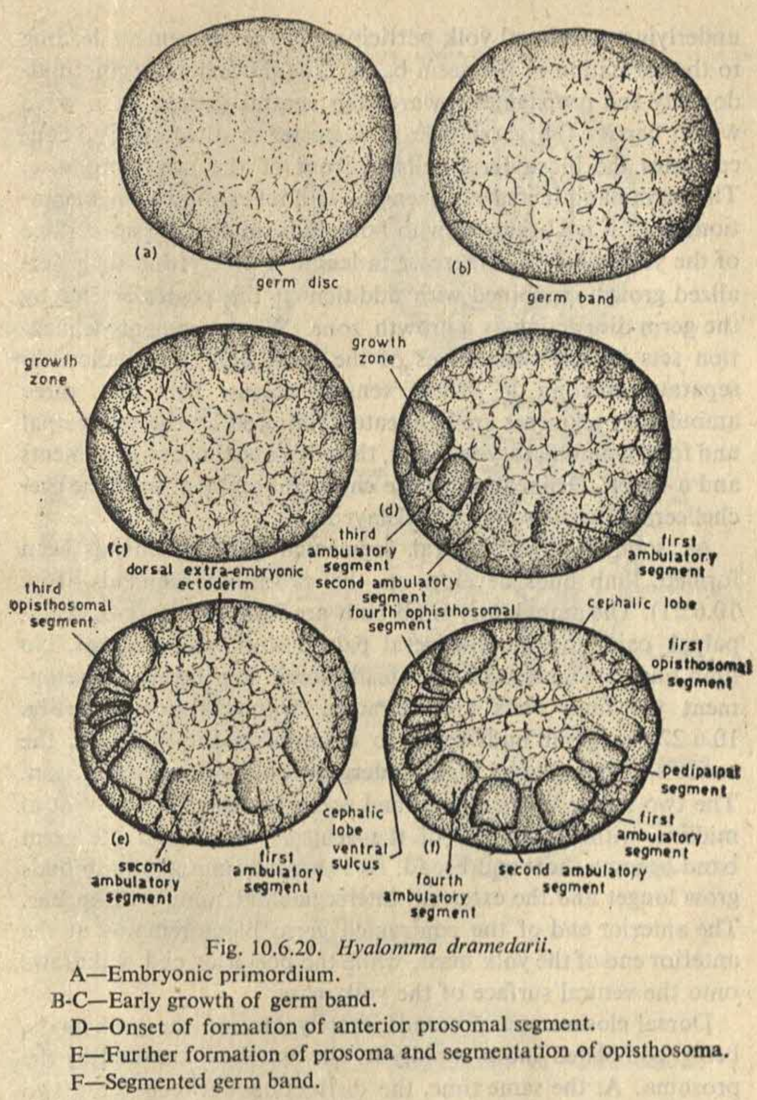

Fig. 10.6.20. *Hyalomma dramedarii.*

A—Embryonic primordium.
B-C—Early growth of germ band.
D—Onset of formation of anterior prosomal segment.
E—Further formation of prosoma and segmentation of opisthosoma.
F—Segmented germ band.

centre. The embryos do not develop a posterior cumulus. Instead, the germ disc as a whole migrates in a posterior direction over the surface of the yolk mass and a broad germ band is formed by the aggregation and proliferation of blastoderm cells in front of the germ disc. The entire blastoderm and underlying peripheral yolk participate in the movement leading to the formation of the germ band. The movement begins mid-dorsally and propagates towards the ventral surface in a wave which carries the germ disc in a posterior direction and concentrates the blastoderm cells in front of the migrating disc. The germ band increases in length, without exhibiting segmentation, until it is U-shaped, with both ends on the dorsal surface of the yolk mass. The increase in length is likely due to generalized growth combined with addition at the posterior end by the germ disc acting as a growth zone. When segment delineation sets in, the two halves of the germ band simultaneously separate creating a broad ventral sulcus. The first three ambulatory segments are delineated, followed by the pedipalpal and fourth ambulatory segment, then five opisthosomal segments and a telson. Delineation of the cheliceral segment from the precheliceral lobes is somewhat delayed.

As soon as the bilateral segmented germ band has been formed, limb buds develop on the prosomal segments (Fig. 10.6.21). The ambulatory limb buds are formed before the pedipalpal pair, and the cheliceral pair is somewhat delayed. No limb buds are formed on the opisthosoma. During later development the limb buds are retracted within the body (Fig. 10.6.22). As limb buds begin to develop on the prosoma, the entire segmented germ band undergoes longitudinal contraction. The two halves of the germ band come together in the ventral midline, with elimination of the ventral sulcus, and the germ band become short and broad. At the same time, the limb buds grow longer and the external, intersegmental annuli disappear. The anterior end of the contracted germ band remains at the anterior end of the yolk mass, while the posterior end withdraws onto the ventral surface of the yolk mass.

Dorsal closure now proceeds directly around the yolk mass, being completed more rapidly by the opisthosoma than by the prosoma. At the same time, the distinction between these two parts of the body is obliterated and a secondary distinction is established between the gnathosoma and histiosoma. As the ambulatory limbs grow longer, extending back beneath the ventral surface of the embryo, the opisthosomal wall spreads dorsally to embrace the posterior part of the yolk mass, and the prosomal wall spreads in similar fashion, carrying the bases of

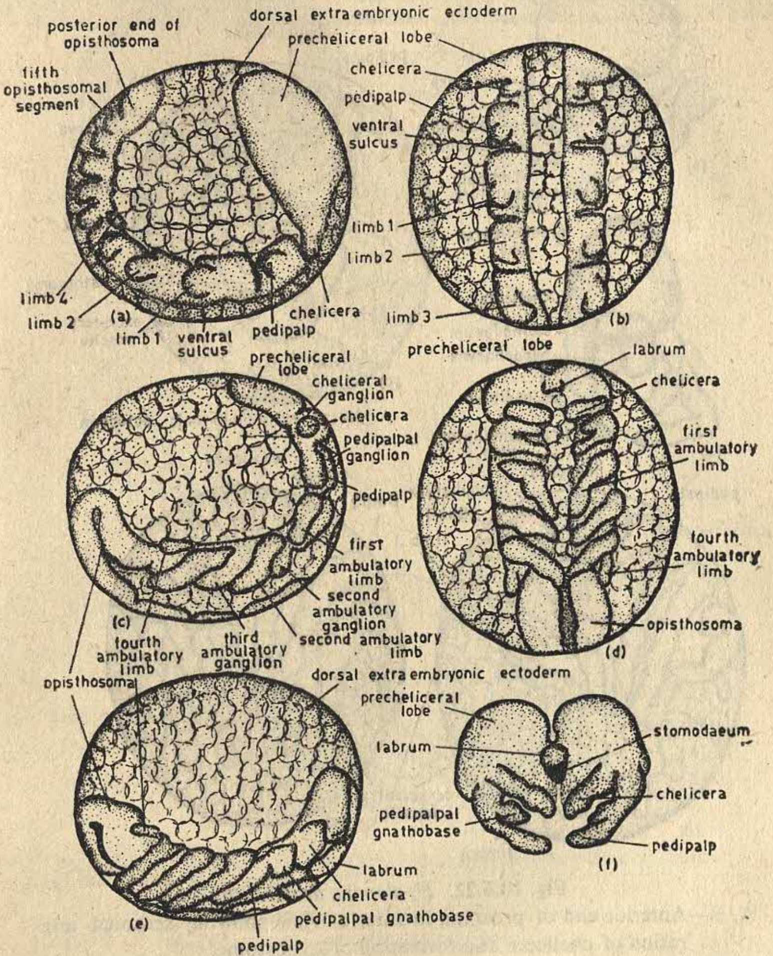

Fig. 10.6.21. *Hyalomma dromedarii*.

A, B—Formation of limb buds, lateral and ventral view.

C-E—Ventral eoncentration of germ band, closure of ventral sulcus, elongation of prosomal limbs.

F—Anterior end of prosoma in anterior view, chelicerae becoming preoral.

the ambulatory limbs to a more lateral position. The completion of the body form is thus attained without any movement of the yolk mass relative to the body wall.

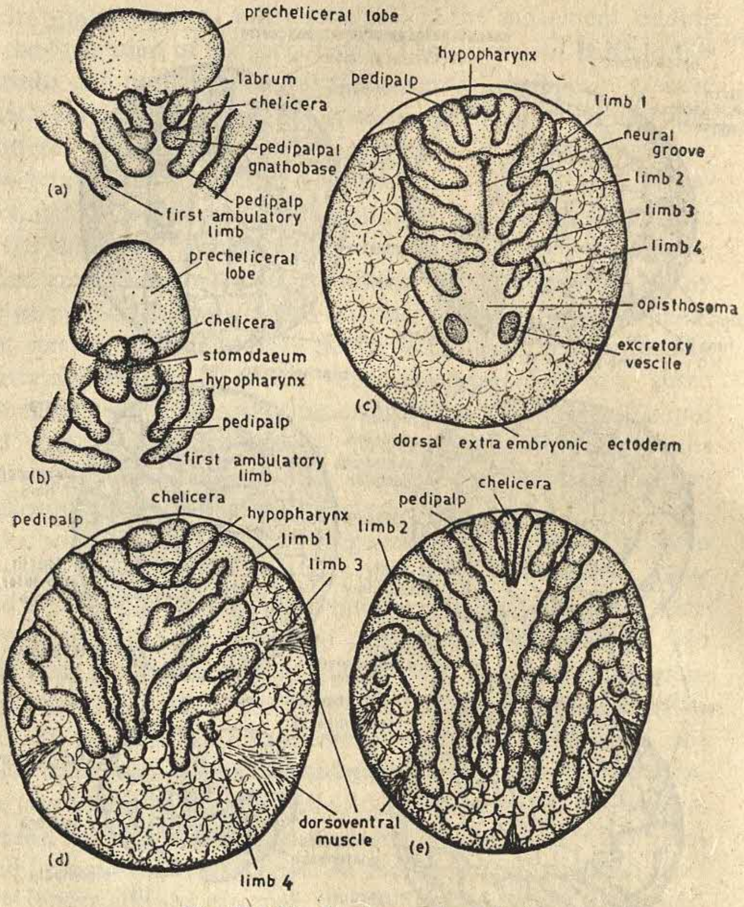

Fig. 10.6.22. *Hyalomma dromedarii.*

A, B—Anterior end of prosoma in anterior view showing seasonal migration of chelicera and formation of hypostome.

C—Shortened germ band.

D—Embryo during dorsal closure.

E—Embryo approaching hatching.

During contraction of the germ band (Figs. 10.6.21, 10.6.22) a median labral lobe arises in the midline between the precheliceral lobes. Simultaneously the chelicerae and pedipalps increase in length and a median lobe develops on the coxa of each pedipalp. The chelicerae then migrate forwards to a preoral position. The bases of the chelicerae come together in front

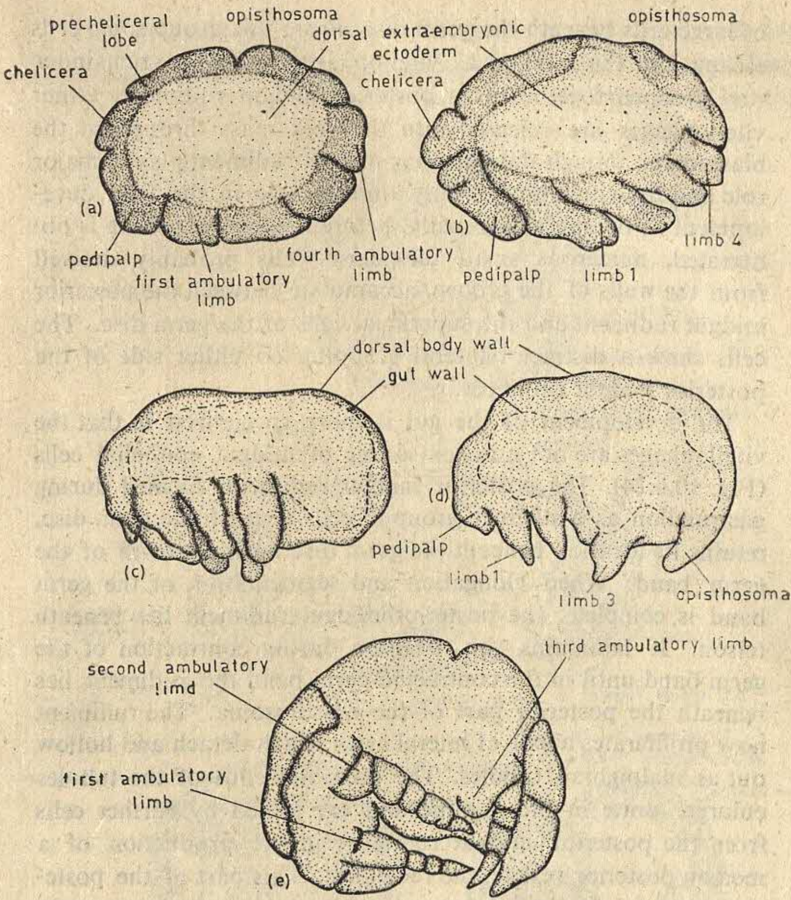

Fig. 10.6.23. The external features of development of *Pediculopsis gramiuum*.
A—Segmented germ band.
B—Formation of prosomal limb buds.
C—Dorsal closure.
D, E—Completion of external development.

of the mouth, with elimination of the labrum. Behind the mouth, the median coxal lobes of the pedipalps come together to form the hypostome, while the remainder of each pedipalp forms a functional palp. Semilunar grooves are lacking on the precheliceral lobes.

It was observed that the gastral grooves is the site of proliferation of cells into the interior, initially forming a compact group of large cells beneath the germ disc. From this group some cells escape into the interior as vitellophages while the remainder stay grouped together as a posterior midgut rudiment. Other vitellophages are released into the yolk mass throughout the blastoderm, though the posterior midgut rudiment plays a major role and the vitellophages only a minor role in the later development of the midgut. Finally, before the gastral groove is obliterated, numerous small mesoderm cells probably derived from the walls of the groove, accumulate between the posterior midgut rudiment and the superficial cells of the germ disc. The cells show a distinct bilateral grouping on either side of the posterior midgut rudiment.

The development of the gut is more specialized in that the vitellophages are not a major source of midgut epithelial cells (Fig. 10.6.24). The posterior midgut rudiment, formed during gastrulation as a distinct group of cells beneath the germ disc, retains its location beneath the germ disc during growth of the germ band. When elongation and segmentation of the germ band is complete, the posterior midgut rudiment lies beneath telson. It maintains this position during contraction of the germ band until in the contracted germ band the rudiment lies beneath the posterior part of the opisthosoma. The rudiment now proliferates a pair of lateral arms which detach and hollow out as malpighian tubules. The proximal ends of the tubules enlarge, unite in the midline and are joined by further cells from the posterior midgut rudiment in the production of a median posterior vesicle, the rectal sac. This part of the posterior midgut rudiment is obviously equivalent to the entire posterior rudiment of spiders. The rectal sac establishes the usual connection with the proctodeum but remains separate from the functional midgut sac. The remaining cells of the posterior midgut rudiment are the major source of anterior midgut cells. Towards the end of the embryonic development, these cells proliferate and spread around the yolk mass to enclose the yolk and the vitellophages. The mesoderm presses into the side of the midgut sac, dividing it into several pairs of diverticula. The stomodaeum gives rise to the lining epithelium of the pharynx and oesophagus.

The mesoderm forms paired bands which break up into somite rudiments, but the formation of coelomic spaces within the somites is relatively vestigial. There are six prosomal and five opisthosomal pairs, the pre-cheliceral mesoderm being diffuse. Each prosomal somite sends the usual appendicular lobe into the corresponding limb bud. After contraction of the germ band the somites break down and develop as limb musculature and dorsoventral somatic muscles.

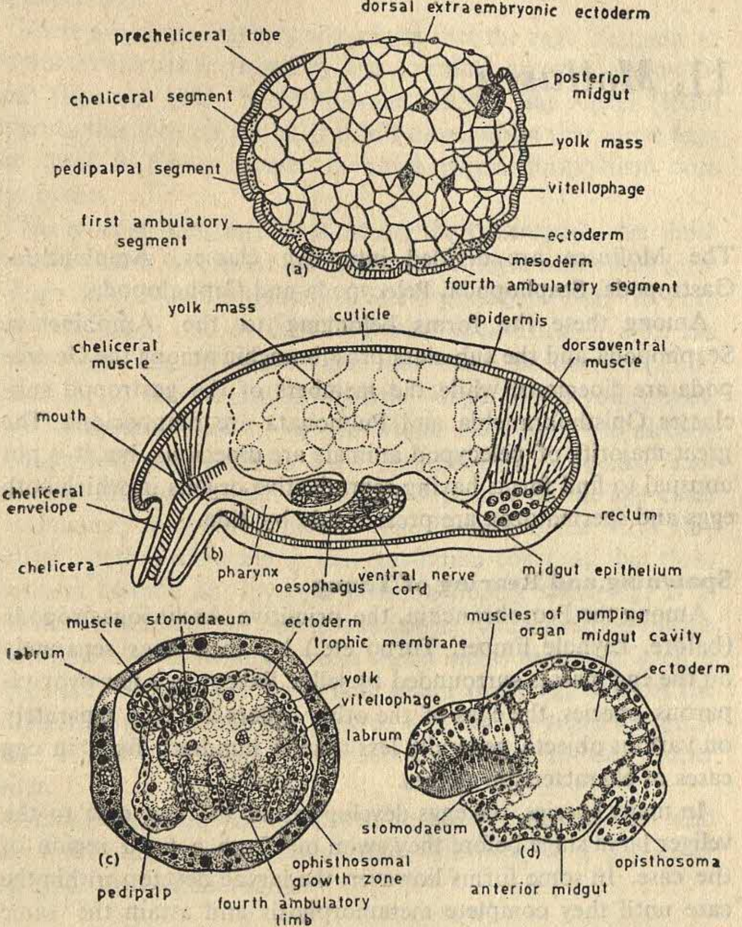

Fig. 10.6.24. A—S.S. through segmented germ band stage of *Ornithodorus* showing vitellophages and posterior midgut rudiment.

B—S.S. through late embryo of *Ornithodorus*.

C—Lateral view of embryo of *Pselaphocherues* at onset of growth of opisthosomal rudiment.

D—S.S. through the embryo of *Neobisium* as opisthosomal proliferation proceeds.

11. *Mollusca*

The Mollusca are divided into five classes, Amphineura, Gastropoda, Scaphopoda, Pelecypoda and Cephalopoda.

Among these the forms belonging to the Amphineura, Scaphopoda and the sub-class prosobranchia among the Gastropoda are dioecious, while the members of the gastropod subclasses Opisthobranchia and Pulmonata are monoecious. The great majority of pelecypod animals are dioecious, but it is not unusual to find forms having reproductive organs in which both eggs and spermatozoa are present side by side.

Spawning and Rearing of Young

Among the Prosobranchia, the primitive Archaeogastropods (balore, keyhole limpet, Turbo etc.) lay their eggs separately on the egg masses surrounded by jelly. Except in a few ovoviviparous species, the eggs of the other orders are laid separately on various objects, generally less than a hundred, fixed in egg cases of characteristic shapes.

In many species, the eggs develop inside the egg case to the veliger larva stage before they swim out from a fixed region of the case. In some forms however, the larvae develop within the case until they complete metamorphosis and attain the same morphology as the adult before they come creeping out.

It is thought that the embryos utilize the material within the case as a source of nutrition, there are also many forms in which some of the embryos grow by digesting others within the same case. For example in *Lucella lapillus*, although several hundred eggs are laid, only 15-25 hatch. This phenomenon is often seen among the Muricidae, Buccinidae and others, in which unfertilized and polyspermic eggs and embryos which stop developing during the early stages are used as food by the veliger larvae. In these species the larvae hatch after passing through the veliger stage, and their size varies with the number of nurse eggs.

There are some workers who claim that the eggs destined to be nurse cells are fertilized by the so-called apyrene spermatozoa (Hyman 1923, 1925; Portman, 1931). Bat Aukel (1930) opposes this idea on the basis of his observation that nurse eggs are found in *Natica catena*, in which sperm dimorphism does not occur.

The most striking egg cases among the Gastropoda are those found in some species of the genus *Cymbium* in which they form a huge mass 35 cm long, shaped like a pineapple with the core removed. Each one of these egg cases measuring 1.5×2.5 cm encloses one egg. Within a single such mass the developmental stages, from the earliest through the veliger to the young adult measuring more than 1.5 cm, in which the velum has already disappeared, can be found lined up in regular sequence. This indicates that one animal must have deposited all the egg cases continuously over a long period. The divers on the ships that collect pearl oysters along the Australian coast say that these molluscs assume an inverted position during spawning. It is not known whether the animal maintains such a position during the whole of a spawning process which seems to require such a long time. It is interesting to note that a single egg mass of the animals in this genus will provide a complete series of specimens of the developmental process arranged in the proper order.

Among the pelecypoda, most of the fresh water species and very few of the marine species shelter the young in a particular region of the ctenedia (gills).

Eggs and Fertilization, Artificial and Natural

Molluscan eggs are all fertilized before extrusion of the first polar body, that is during the primary oocyte stage. Among the Gastropoda, copulation and internal fertilization are found in all the Opisthobranchia and Pulmonata. The lower Prosobranchia, Pelecypoda and the Scaphopoda with a few exceptions are characterized by external fertilization.

Even among the animals showing external fertilization, there are only a few species in which it is possible to observe the phenomenon of fertilization and the developmental processes by using eggs and spermatozoa from extirpated gonads. In most species it is necessary to wait for natural spawning or use a variety of methods to induce shedding. If egg water is used as the stimulus, the sperms are expelled together with a column of water two metres high (Wada, 1954).

The species among the Gastropoda in which fertilization can be carried out artificially are extremely few, consisting only of the Fissurellidae, which include *Megathura crenalata* and *Scutus scapha*. In some cases artificial fertilization has been successful in the scaphopod *Dentalium* spp.

In most cases the mature eggs are spherical or nearly so, and the eggs removed from pelecypod ovaries are often drawn to a point at one side. This pointed region is the part which was attached to the gonadal epithelium and marks the vegetal pole of the egg. The egg of *Fulota similari stimpsori* has a very peculiar structure (Fig. 11.1). As the egg of this species approaches maturity, the cytoplasm forms many long protruberances, at the tip of which the spermatozoa are said to enter.

When the eggs are surrounded by a secondary membrane (chorion), it has an umbilicus like process. There are some who consider this structure to be a micropyle but this is incorrect, since spermatozoa are observed to enter through any part of the chorion.

In the Amphineura are to be found species in which the chorion assumes a highly characteristic structure (Fig. 11.2).

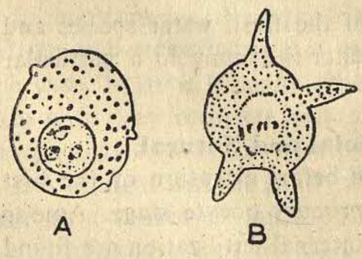

Fig. 11.1. Two stages in the maturation of the *Eulota similaris stimpsoni* egg.
Protrusions through which the sperm enter the egg are formed as it matures.

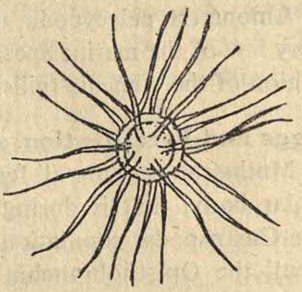

Fig. 11.2. Egg of *Liolophura japonica.*

Among the Prosobranchia, in some species of the order Archeogastropoda having eggs with chorion, it is known that the spermatozoa undergo an acrosome reaction which is believed to enable the spermatozoa to dissolve the chorion and enter the perivitelline space. It has been found that a striking change takes place in the acrosome region of a large number of pelecypod spermatozoa at the time of fertilization. Since the eggs are small, it is relatively easy to observe the fertilization process in pelecypod eggs. After sperm attachment several minutes are usually required for the sperm head to disappear inside the egg. The sperm tail is taken in also. Unlike echinoderm fertilization no sudden change in the nature of the vitelline membrane has been determined to take place immediately after molluscan fertilization, although in pelecypod eggs the space between the vitelline membrane and the plasma membrane becomes a little wider after fertilization. There is a record that molluscan spermatozoa enter the eggs from the vegetal pole, but no fact of this nature can be confirmed in *Crassostrea gigas*, *C. echinata*, *Mactna veneriformis* or *Mytilus edulis*.

After fertilization, the first and second polar bodies are extruded successively at the animal pole. The time from fertilization to first polar body extrusion is greatly affected by the condition of the egg nucleus at the time of fertilization. The first polar body usually does not cleave again, although there are reports of its division. In *Crassostrea echinata* it often cleaves when the vitelline membrane is weakened. The second polar body is not as conspicuous as the first in pelecypod eggs. At the time of polar body formation not only the animal pole of the egg but also the vegetal pole undergoes a change in form in many cases.

The sperm lies in the egg cytoplasm near the surface without much change in position until the expulsion of the second polar body. When the maturation divisions of the egg are completed the egg and sperm pronuclei come together near the centre of the egg and unites in syngamy and with this the first cleavage begins.

Early Development

TERMINOLOGY OF CLEAVAGE

The molluscan eggs belong to the category of so-called 'mosaic eggs'. The cleavage is of spiral type. In order to describe the process of development it is convenient to designate each blastomere by an appropriate term. The formula in general use is that used by Conklin (1897) to describe the development of *Crepidula*. Taking the 4-cell stage as the standard, the blastomere as seen from the animal pole side are designated A, B, C and D in clockwise order. Since in most cases these blastomeres are larger than those formed later, they are also called macromeres. (The designations of the 2-cell stage blastomeres, AB and CD are formed from those of the 4-cell stage.) In general, the two opposite blastomeres A and C are in contact at the animal pole surface, while, B and D are in contact at the vegetal pole (Fig. 11.3). The four micromeres which separate off toward the animal pole side from these large blastomeres are called a quartet and labelled with the small letters a, b, c and d. To these are added a numerical coefficient identifying their generation as the first (1a, 1b etc.) and differentiating it from other quartets. Sometimes a quartet is designated collectively by the letter 'q'. Since the quartets are formed from the macromeres in the course of several cleavages, each quartet receives a coefficient, specifying its generation. When the blastomeres making up a quartet divide again, the member of the two resulting blastomeres lying on the animal pole side receives the index number 1, while its sister blastomere towards the vegetal pole receives the index number 2. Thus when the first quartet cleaves, it produces $1q^1$ and $1q^2$. At the next cleavage $1q^1$ produces $1q^{11}$ and $1q^{12}$. Conklin retained the large letters A, B, C and D for the four macromeres which lie together at the vegetal pole. Blastomeres which are destined to play a special role in the future are also sometimes given special notation. For example the 4d blastomere is called M or ME. In pelecypod development, the 2d blastomere is designated as X.

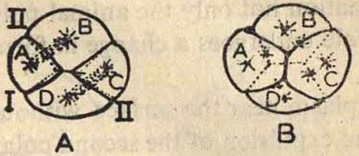

Fig. 11.3. Cleavage in *Trochus*. A-B—4 cell stage (from animal pole.)

Among the Amphinura, the development of the Loricata (chitons) has been investigated by Metcalf (1873), Kowalevsky (1883) and especially Heath (1899) working with *Ischnochiton* *magdalensis*. The mode of development of these animals is found to agree extremely well with that of the gastropoda. A great deal of research has been done on the Gastropoda.

The Pelecypoda make up the next widest group after the Gastropoda. The number of species is also large, and a good many people have published observations of a general nature concerning the development of these animals. Observations on the Scaphopoda have been made by Wilson (1904), Kowalevsky (1883), Schleip (1925) and others. In general the early development of these species agrees well with those of the Gastropoda and other groups.

MODE OF CLEAVAGE

Cleavage in the Amphinura, Gastropoda, Scaphopoda and Pelecypoda is total, and follows the spiral cleavage pattern. Among the Amphineura and Gastropoda, except for a few species, in the latter group which form a polar lobe (or yolk lobe), the first two cleavages result in four practically equal sized blastomeres. Indications of spiral cleavage are already present at the second cleavage, in that the A and C blastomeres of the four cell stage are in contact at the animal pole, and B and D at the vegetal pole (Fig. 11.3).

Even in the species of Pelecypoda which do not form polar lobes the first cleavage is unequal, dividing the egg into a smaller AB and a large CD blastomere. The first cleavage plane, while passing through the animal pole, does not include the egg axis, but makes a certain angle with it. In other words, the material of the vegetal pole is all contained in the CD blastomere. The next cleavage takes place in a spiral manner similar to that in the gastropods, forming equally sized A, B and C blastomeres and a large D blastomere. In the *Mactna* egg, CD often cleaves considerably ahead of AB.

In the gastropods, *Nassa*, *Nassarius*, *Ilyanassa* and *Aplysia*, the scaphopod, *Dentalium* and the pelecypods, *Crassostrea*, *Pinctada*, *Pteria*, *Mytilus*, *Spondylus* and others, a polar lobe is formed during the early cleavages. As the mitotic apparatus for the first cleavage develops in these eggs, the vegetal side bulges out. As the cleavage furrow is formed, the egg is apparently divided into three nearly equal sized parts (Fig. 11.4). This vegetal bulge is the first polar lobe—this particular stage is called as *trefoil stage*. There is no nuclear substance in the polar lobe. The first polar lobe is attached to one of the two blastomeres of the animal pole side and eventually its contents flow into this blastomere. The two-cell stage is thus made up of a large CD blastomere containing the material of the polar lobe and a small AB blastomere which includes none of this vegetal substance. At the beginning of the next cleavage a polar lobe (second polar lobe) is again formed at the vegetal side of the CD blastomere. This unites with the D cell at the conclusion of the cleavage process. The four-cell stage thus consists of the three practically equal sized blastomeres, A, B and C and a blastomere rather larger than these.

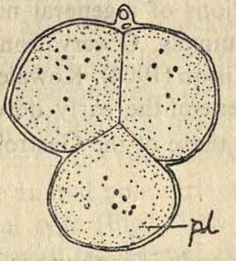

Fig. 11.4. Cleavage in *Pinctada maxima*, formation of first polar lobe (trefoil stage).

A less conspicuous bulging of the vegetal side is often found accompanying the formation of the polar bodies. In the Pelecypoda the polar lobe appears until the second cleavage and is not formed at later cleavage, but in *Ilyanassa* and *Dentalium* it appears also at the third cleavage. In *Dentalium* part of the polar lobe substance is transferred to the 2d blastomere at the fourth cleavage.

Differentiation of Germ Layers

SEGREGATION OF ECTODERM

In the course of the three cleavages following the four cells stage, all the material to form the cells of the ectoderm in separated off from the four macromeres. At the first of these cleavages the micromeres are produced in a dextronotational direction, as is characteristic of spiral cleavage. These four blastomeres, that is, micromeres 1a, 1b, 1c and 1d form the first quartet. They may be referred to collectively by the abbreviation 1q. These cleavages do not necessarily occur simultaneously.

The next cleavage of the macromeres (1A-1D) is sinistral, giving forth the second micromeres quartet (2q, 2a-ad). In the

Gastropoda these micromeres are smaller than the macromeres and in especially yolky eggs size differences may be extreme (Fig. 11.5). In the Pelecypoda the 2d micromere is larger than the 2D macromere, and is, in fact, the largest of the blastomeres which make up the embryo at this stage. The 2d blastomere is homologous with the first somatoblast which is seen in annelid development. In the Pelecypoda it later produces the ventral plate, shell gland and other ectodermal organs, and Meisenheimor gave it the name of X blastomere (Fig. 11.6).

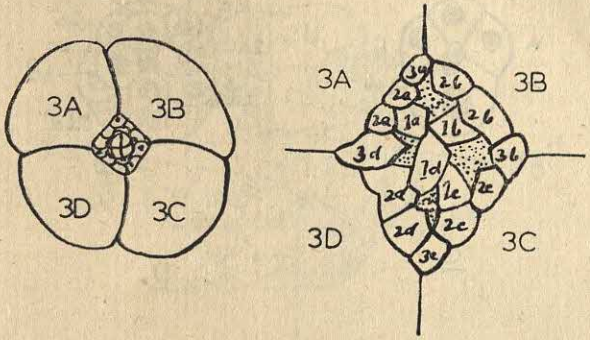

Fig. 11.5. Egg of *Fulgur carica* at 24-cell stage.
A—From animal pole.
B—Enlarged sketch of animal pole region.

Following the separation of the second quartet (2q), the cells of 1q divide, forming the 16-cell stage. This cleavage is also sinistral producing $1a^1-1d^1$, which occupy the animal pole region and the four blastomere, $1a^2-1d^2$, which lie directly above the macromeres, held between the blastomeres of the 2q quartet. These four cells ($1a^2-1d^2$) are called the primary trochoblasts or turret cells, and later produce the velum. In *Trochus* these cells continue to cleave, but in *Fiona* their cleavage is suspended until the 60-cell stage.

When the embryo as a whole is proceeding from the 16-cell to the 32 cell stage the cleavage of each blastomere takes place in a dextral direction although these cleavages are not simultaneous; *Trochus* for example, passes through a 20-cell stage (cleavage of the four macromeres); a 24 cell stage (cleavage of 2q); a 32-cell stage (cleavage of Iq). In the embryo of this stage

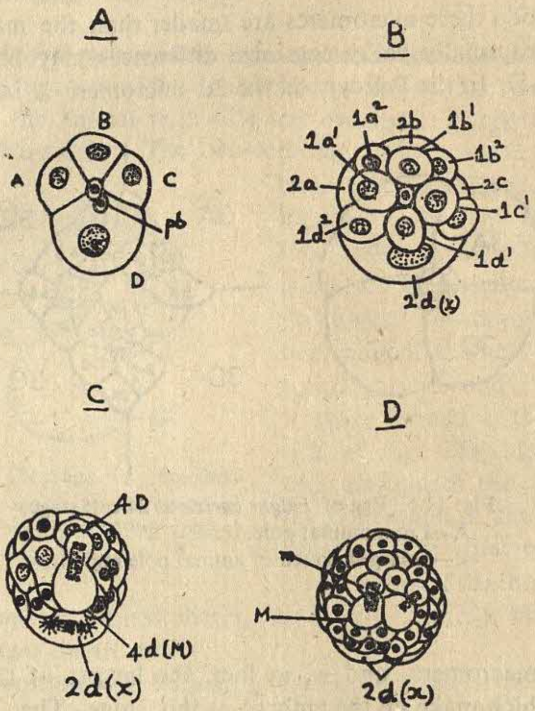

Fig. 11.6. Cleavage in *Dreissenia polymorpha*.
A—4 cell stage.
B—16 cell stage.
C—46 cell stage.
D—mesoblasts (M) cleaving from mesomeres (m)
2d(x)—primary somatoblasts.

the blastomeres are regularly arranged so that four of the daughter cells (1a^{11}—1d^{12}) derived from the cleavage of 1a^{1}—1d^{1} occupy the animal pole, while the positions of the other four (1a^{12}—1d^{12}) lie alternately with these forming the molluscan cross (Fig. 11.7). When Ia^{11}—Id^{11} divide again, they form 1a^{111}—1d^{111}, the apical cells or rosette cells, are 1a^{112}—1d^{112}, the peripheral rosette cells (Fig. 11.7). The peripheral rosette cells correspond to the "annelidan cross" of annelid embryos. The tip cells at the ends of the crossing arms in molluscan cleavage are formed from the 2a^{11}—2d^{11} cells rather than from the first quartet.

Gastropod embryos maintain a radial symmetry up to this stage. They are rather flattened and a small blastocoel appears

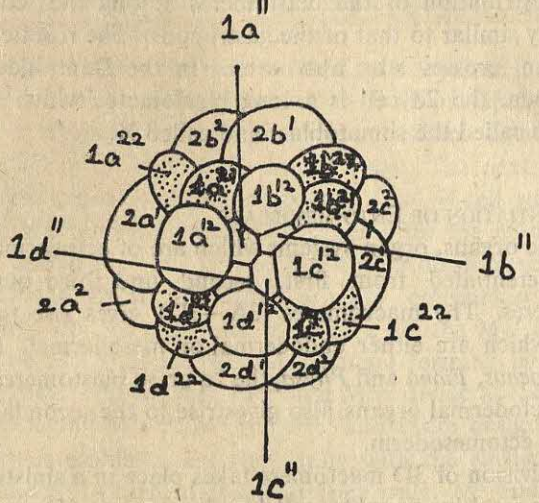

Fig. 11.7. Cleavage in *Trochus*.
A—32 cell stage (from animal pole).
B—63 cell stage (from animal pole).

inside the cell mass. The polar furrow is clearly seen on the surface of the vegetal pole. Along with the polar bodies, the polar furrow forms a good indicator of the embryonic axis. During the course of the three cleavages of the macromeres which leads to the 32-cell stage, the ectodermal cells which will form the ectoblast get separated off from the macromeres. The macromeres (3A—3D) contain all the material of the entoblast and most of the mesodermal material (A small part of the mesoblast is contained in the 2q or sometimes the 3q cells).

Because of the appearance of the large 2d blastomere and its subsequent cleavage in the Pelecypoda, the transition from radial to bilateral symmetry appears sooner than in the Gastropoda. Since size differences occur among pelecypodan blastomeres within the quartets and their cleavage does not occur simultaneously, the cells making up the embryo are not arranged in regular order and the rosette and molluscan crosses do not appear. The organs derived from each quartet are however the same as in the Gastropoda, indicating that the difference in blastomere arrangement is not a fundamental one.

The distribution of the blastomeres among the chitons is extremely similar to that of the gastropods. The rosette and the molluscan crosses are also seen. In the Dentaliidae as in Pelecypoda, the 2d cell is a long blastomere which in both groups is called the somatoblast and called X.

DIFFERENTIATION OF ENTO-MESOBLAST

All the organs, organ systems which are of ectodermal nature are differentiated from first, second and third quartets of micromeres. The macromeres (3A—3D) gives rise to all the organs which are either endodermal or mesodermal. *In Crepidula, Trochus, Fiona* and *Physa*, the 2q or 3q blastomeres besides giving ectodermal organs also gives rise to the secondary mesoderm or ectomesoderm.

The division of 3D macromere takes place in a sinistral direction. The resulting daughter cell 4d is larger than 4D macromeres and eventually sinks into the blastocoel. The 4d blastomere is called the primordial mesoderm cell or mesoblast since part of the entoderm also sometimes differentiates from it, it is also called as mesentoblast or it may be described as the second somatoblast. It may be designated as M or ME. *In Crepidula* and *Fiona*, the cleavage of the 3D blastomere takes place immediately after the embryo reaches the 24-cell stage, preceding the cleavage of 3A, 3B and 3C. In the gastropods which form equal sized blastomeres at the 4-cell stage, it is at first impossible to distinguish between A, B, C and D, but for the first time at the 24-cell stage, the correct designations can be assigned to each blastomere. In *Trochus* the formation of 4D occurs in the 64-cell stage, lagging behind the cleavage of 3A, 3B and 3C.

These three blastomeres also cleave, very unequally giving rise to the 'micromere' 4a, 4b and 4c which are larger than the 'micromeres' 4A-4B and 4C. These micromeres are sometimes called entomeres or secondary micromeres. Together with the micromere of 5q they form the entodermal organs (digestive system). The 4d or ME cell, which has moved into the blastocoel, very soon divides equally and the paired daughter cells take a position in contact with embryonic body wall to the right and left of the median plane. These cells ME^1 (right) and

Me^2 (left) function as teloblasts of the mesodermal bands which constitute the rudiments of mesoderm.

The stage at which the mesentoblast cells separate into mesoblast and entoblast, differ rather widely from one species to another. For example in the opisthobranch *Fiona* 4d-ME arises from the 3D at the end of the 24-cell stage. The next cleavage of 4d takes place in the latter part of the 44 cell stage. The resulting ME^1 and ME^2 cleave together after the 68-cell stage and give rise to Me^1 and E^1 and Me^2 and E^2 blastomeres respectively. E^1 and E^2 are called the primary enteroblasts. These blastomeres do not cleave for some time but the Me^1 and Me^2 cells divide very soon (at the 88-cell stage) forming m^1z^1, M^1e^1 and m^2z^2, M^2e^2 respectively. M^1e^1 and M^2e^2 divide at the 128 cell stage, producing M^1, e^1, M^2 and e^2 e^1 and e^2 are designated the secondary enteroblasts, and they unite with the earlier formed pair E^1 and E^2 to form a group of four cells. M^1 and M^2 are called the mesoblastic teloblasts. Eventually they divide to give rise to two irregular rows of mesoderm cells—m^1z^1 and m^2z^2 cleave immediately after M^1e^1 and M^2e^2 forming m^1, z^1, m^2 and z^2. m^1 and m^2 follow the same course as M^1 and M^2 joining the latter in forming the rudiment of mesoderm. It is said that in *Fiona* the enteroblast cells which arise from the mesentoblast form most of the gut while the z^1 and z^2 cells take care of forming its anteriormost part.

In Crepidula, the segregation of the entoblast takes place earlier than in *Fiona*. Me^1 and Me^2 cells divide, producing the cells m^1 and m^2 which contain only the mesodermal constituents of the parent cells. This show the close connection that exists between mesoderm and entoderm.

In some animals the segregation of the mesoblast takes place even earlier. Lillie (1895) concluded that in *Unio* the cells derived from the 4d blastomere are exclusively mesodermal in nature. The same condition was found in *Dreissensia* by Meisenheimer (1901). In the pulmonate, *Planorbis* the cells derived from the 4d blastomere are said to be all mesodermal (Holmes 1900).

Ecto-mesodermal cells: Lillie (1895) while studying the pelecypod *Unio*, first discovered that among the Mollusca, in addition to the mesoderm which originates from entodermal cells, there also exists mesoderm of ectodermal origin. Lillie found that one cell derived from the cleavage of the 2a micromere of the second quartet falls into the blastocoel and the cells that it later produces form the muscular system of the glochidium larva. From then on observations of the same sort have been made in various molluscan species. This ectodermal mesoderm is called ecto-mesoderm or since it takes part only in the formation of larval organs, larval mesoderm.

Conklin (1897) showed that in the prosobranch *Crepidula*, the ectomesoderm has its origin in three of the 2q blastomeres (2a, 2b, 2c). In the opisthobranch *Fiona* this rudiment can only be seen after gastrulation has already begun. The cells $3a^{211}$ and $3a^{221}$, and also $3b^{211}$ and $3b^{221}$ divide as they sink into the blastocoel; $3a^{2112}$, $3a^{2212}$ and $3b^{2112}$, $3b^{2212}$ are pushed out toward the blastopore, while the large daughter cells are gradually covered over by the growing ectoderm. These cells $3a^{2111}$, $3a^{2211}$, $3b^{2111}$ and $3b^{2211}$ are the rudiments of the ectomesoderm. These divide later, each forming a band composed of several cells which lie at first in the anterior part of the gastrula, but are later found in the head region of the larva. In the pulmonate *Physa* also, completely identical cells form the ectomesodermal rudiment (Wierzejski, 1897). In *Planorbis* again eight cells (2111, 2112, 2211 and 2212 of 3d and 3c) are said to be the ecto-mesomeres (Holmes, 1897).

The fact that in the Mollusca the ecto-mesoderm is seen only during the larval period, disappearing at the time of metamorphosis, is highly interesting from the phylogenetical point of view.

Blastula to Trochophore Larva

BLASTULA

In the Mollusca the blastocoel is formed very early in the eggs where there is relatively little yolk. In general on account of the large size of the entomeres (entoderm forming blastomeres, entoderm cells) which lie at the vegetal side, the blastocoel is very small when compared with those of echinoderms and other blastula. This is particularly true of eggs with a large amount of yolk which form typical stereoblastulae.

The embryo in the blastula stage develops cilia at certain regions on its body surface. At the animal pole, on the apical cells, apical cilia are formed. On the sides of the blastula at four points on the primary trochoblasts, four clumps of cilia are formed. These resemble the ctenophore comb-plates (Fig. 11.7). As development proceeds, certain cells in the vicinity of the primary trochoblasts which are called the secondary trochoblasts also produce cilia. All these cilia form a complete ring of cilia, which is called the prototroch. In many species especially Pelecypoda, the apical cilia develop to become very prominent to the extent that they are often described as a 'tuft of flagella'. In most of the species these tuft of flagella generally persist through the veliger stage, but in *Crassostrea* they are absent from the beginning. In the case of pearl oysters they disappear early in the veliger stage.

After the appearance of these cilia, the embryo begins to swim gracefully through the water or move slowly within the egg case. In the species in which the eggs are surrounded by a chorion, the embryos escape through the so-called chorion micropyle. The larvae of oysters, common mussels and many other pelecypods begin their swimming life with the vitelline membrane intact and shed it slowly bit by bit.

GASTRULATION AND GASTRULA STAGE

In the case of Mollusca gastrulation takes place by emboly as well as by the common method, the epiboly. In *Crepidula* where typical stereoblastula exist, typical epiboly can be seen (Fig. 11.8). The genus *Paludina* shows typical emboly (Fig. 11.9). There are many species which follow a process that might be called intermediate between these two. As early as the

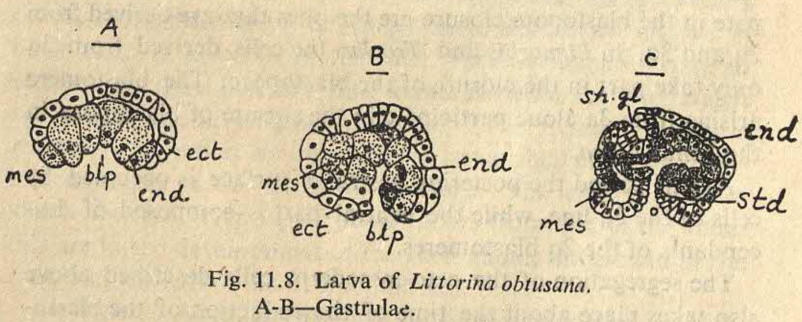

Fig. 11.8. Larva of *Littorina obtusana*.
A-B—Gastrulae.
C—Larvae with shell gland.
blp—blastopore; ect—ectoderm; end—endoderm; mes—mesoderm; o—mouth; sh.gl—shell gland; std—stomodaeum.

30-cell stage in *Patella*, some of the cells of the vegetal pole region migrate into the blastocoel. This is thought to constitute the beginning of gastrulation (MacBride, 1914). In general, however, it begins later than this.

From the beginning of gastrulation, the cells arising from the 2q, especially those derived from 2d, proliferate actively in the dorsal part of the embryo. As a result, the entodermal cells which have been located near the vegetal pole are shifted toward the ventral side. The embryo thus becomes flattened dorso-ventrally, and with the invagination of the ectoderm cells to form the shell gland, assumes a well defined bilateral symmetry.

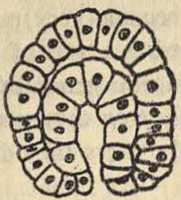

Fig. 11.9. Gastrula of *Paludina vivipara*

Invagination in the pelecypod *Preissensia* occurs by a process of emboly while in the oysters and many other marine bivalves the blastula lacks a blastocoel. In these cases the invagination begins as an overgrowth (epiboly). Very shortly, proliferation of the ectoderm cells causes a blastocoel to form and the mode of invagination changes over to that of emboly.

In the beginning as a result of the entomere invagination a wide blastopore is seen but it becomes gradually reduced to a long, narrow longitudinal slit by the proliferation of the 2q and 3q cells. In many species the blastopore becomes completely closed. In *Crepidula* and *Ischnochiton*, the cells which participate in the blastopore closure are the ones that are derived from 2q and 3q. In *Planorbis* and *Trochus* the cells derived from 3q only take part in the closure of the blastopore. The blastomere arising from 3a alone participate in the closure of blastopore in the genus *Fiona*.

At this period the posterior gastrular surface is occupied by cells of the 3q line, while the central part is composed of descendants of the 2q blastomeres.

The segregation of the ecto-mesoderm cells described above also takes place about the time of the reduction of the blastopore (*Fiona*). In the Gastropoda a large cell which will form the excretory organ of the future larva can be seen on the right side in the posterior part of the embryo.

At the time when the archenteron becomes clearly defined the cells composing it are the macromeres 5A, 5B, 5C and 5D lying farthest inside. Next to them are the 5a, 5b and 5c cells and above these $4c^2$, $4b^2$, $4a^2$ and $4c^1$, $4b^1$, $4a^1$, lie directly below the ectoderm. In the posterior part of the body can be seen the enteroblasts E^1, E^2, e^1, e^2 which have been derived from 4d.

Accompanying the development of the archenteron and the contraction of the blastopore, the ectoderm around the latter gradually invaginates, forming the stomodaeum in this position. That is, the future mouth occupies the position of the blastopore but among the Gastropoda there are some species in which the blastopore is closed and a new mouth opening is formed (*Crepidula*, *Trochus*, *Fiona*, *Nassa*). In others the blastopore remains open and changes over to a mouth (*Patella*, *Limnaea*, the Pteropoda and the Heteropoda). In this connection *Paludina* forms a noteworthy exception. In this case the blastore changes into the anus and a new mouth is formed in a different position.

TROCHOPHORE AND VELIGER STAGES

With the exception of the Cephalopoda, molluscan larvae develop from the gastrula stage into a form which closely resembles the annelidan trochophore larva. This is also called a trochophore. In the molluscs the prototroch of the trochophore larva eventually develops prominently to form an organ of locomotion called the velum. A shell also forms and the larva takes on the characteristics of the veliger stage. No drastic change in body form is involved in the shift from trochophore stage to veliger and the boundary line drawn between the two stages varies from one investigator to another. The present description will treat the period lasting until the larval shell becomes a prominent organ at the trochophore stage. The chief points in which the molluscan trochophore differs from that of the annelids are in the development of the shell gland, the growth of the foot, and the formation of a radula sac.

Shell gland: After the invagination of the entoderm, the cells of the opposite side (dorsal) of the body proliferate vigorously and invaginate towards the blastocoel (Fig. 11.8) forming the shell gland. In this stage the embryo thus shows two invaginated parts, the shell gland dorsally and the archenteron on the ventral side. Slowly the shell gland invagination disappears, and the flattened tissue of the gland begins to secrete the larval shell. It has been observed in *Amphibola* (pulmonate) the shell gland begins to secrete without invaginating, but in other gastropods and the pelecypods, invagination invariably precedes shell secretion. According to Raven (1952) in *Lymnea* the invaginated shell gland cells maintain very close histological contact with the epithelial cells of the midgut. He believes that the shell gland is induced by contact between the gut and the ectoderm, but he has no experimental proof to support this hypothesis. Finally the shell gland is everted, causing a slight bulging of the body wall. As a result of this eversion, the hitherto practically solid embryo acquires a large body cavity.

The shell gland is formed of cells derived from the 2d blastomere. According to Holmes (1900) in *Planorbis* they are derivatives of $2d^{12}$ and $2d^{21}$.

Foot, Pedal gland, Statocyst: One of the characteristics of the molluscan trochophore is that as development proceeds, the anus moves towards the mouth. Between these two, the foot is formed. In *Patella*, *Fulgur*, *Trochus* the foot appears as a pair of protuberances, but in general it is first recognised as a single proliferation. There are some differences among species with respect to which cells form the foot. In *Unio* and *Crepidula* it develops from the 2q cells. In *Planorbis* and *Trochus* from 3q cells. In *Fiona* the derivatives of both these quartets are involved.

In the Gastropoda, the development of the foot lags behind that of the shell gland. But the operculum is secreted on the lower surface of the foot, rather early in the late trochophore stage. The formation of the operculum is peculiar to the gastropods, and even when the adult form lacks an operculum, one develops in the larva. In *Paludina* the foot forms in front of the blastopore, posterior to the point where the future mouth will appear. In the Pelecypoda the foot undergoes practically no development during trochophore stage.

From the Amphineura to the Pelecypoda, all the Mollusca show an invagination of the ectodermal cells to form a pedal gland on the posterior part of the foot (anterior in the Amphineura). On the two sides of the base of the foot there are also ectodermal invaginations which give rise to the statocysts. The statocysts later receive innervation from the brain ganglia. They develop earlier than these ganglia (Gastropoda) and near the statocysts the foot ganglia differentiate from ectoderm cells.

Radula sac: The possession of a radula is one of the most distinctive features of all the Mollusca except the Pelecypoda. The rudiment of the radular sac appears immediately after the invagination of the stomodaeum and constitute one of the distinguishing characteristics of the trochophore stage including that of the Pelecypods. In general the rudiment of the radula sac develops from cells of the stomodaeum which grow and form a pocket extending towards the outside of the stomodaeum posteriorly (Fig. 11.10). According to Wierzejski (1905), in *Physa* it appears as a pair of invaginations behind the mouth but not related to the stomodaeum.

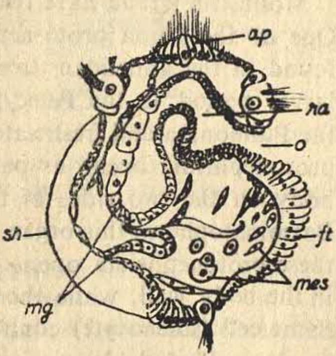

Fig. 11.10. Section of trochophore larva of *Patella.*
ap—apical plate; ft—foot; mes—mesoderm; mg—midgut; o—mouth; ra—radula sac, sh—shell.

Velum: As the trochophore larva approaches the veliger stage, the prototroch increases in size and this prototroch is then called the velum. In *Ischnochiton* and *Trochus* the prototroch is formed by the trochoblast cells, the tip cells of the molluscan cross and others which are derived from 1q and 2q. In some Amphineura and primitive Gastropoda, the prototroch and in turn the velum are more radially symmetrical than those of the members of the other species. The free-swimming period of these larva is extremely short (several hours to several days) and as a consequence the velum is not highly developed. In the other Gastropoda and the Pelecypoda, the free swimming period is prolonged and the velum is well developed. A typical velum is composed of two ciliated rings anterior to the mouth called the preoral velum and the other which develops posterior to the mouth, the postoral velum. In *Crepidula* and *Planorbis* it is said that only $1a^{22}$, $1a^{21}$, $1b^{22}$, $1b^{21}$ among the prototroch cells take part in forming the preoral velum. In addition, the tip cells of the anterior arm and the lateral arms of the molluscan cross are used into the formation of the preoral velum in *Crepidula*, while the postoral velum is mainly composed of 2q cells to which some 1q and 3q cells are added.

LARVAL EXCRETORY ORGAN

Molluscan larvae have two main kinds of excretory organs. One of these is a proto-nephridium of the same sort as that found in the annelidan trochophore, this appears during the larval period in the Pelecypoda and among Gastropoda, in the Pulmonata and freshwater Prosobranchia (*Paludina*). These protonephridia occur as paired organs lying ventrally in the body on the two sides of the foot. In the pulmonate group Bassomatophora, this organ is formed of four cells. One end of these protonephridia opens to the outside through an opening in the body wall, while the other end consisting of a ciliated flame cell (solenocyte) connects with the primary body cavity. In the wall of the protonephridium are excretory cells with a very large nucleus (Fig. 11.11). In the Stylommatophora a large number of cells participate in the formation of the protonephridium. Among the Pelecypoda, it is a straight organ composed

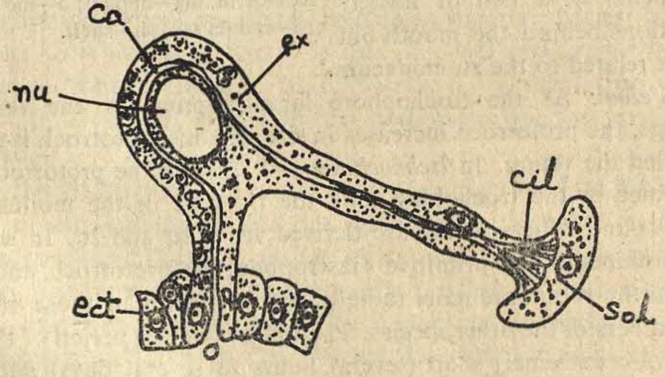

Fig. 11.11. Protonephridium of Bassomatophora.
ca—protonephridial canal; cil—cilia; ect—ectoderm; ex—excreting cell; nu—nucleus of excreting cell; o—opening; sol—solenocyte.

of two or three cells, but the structure is similar to that of the Pulmonata (Fig. 11.12).

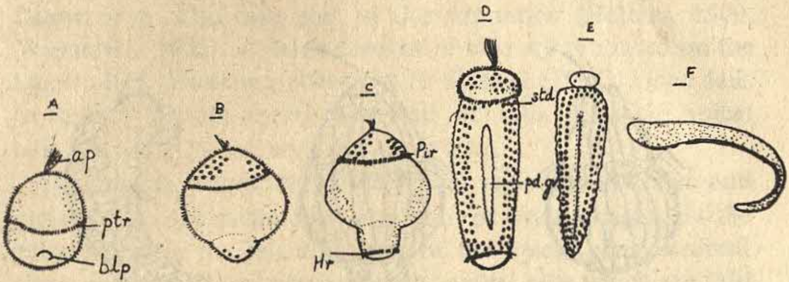

Fig. 11.12. Larval development in *Epimenia verrucosa*.

A—Trochophore larva 20 hours after hatching, stomodaeum invaginating.

B—Trochophore, 24 hours.

C—Trochopore at 2 days. Telotroch appears at posterior end of larva.

D—Tochophore at 4 days. Pedal groove appears.

E—Larva at late metamorphosis stage. Cell being sloughed off from degenerating head and tail regions.

F—Young adult, 19-33 days after hatching.

ap—apical cilium; blp—blastopore; pd-gv—pedal groove; ptr.—prototroch; std—stomodaeum; ttr—telotroch; x—degenerating head region.

Various different opinions have been expressed concerning the question of whether the protonephridium in these species is derived from the ectoderm or from the mesoderm. Concerning the Bassomatophora, Fol (1875, 1880), Wolfson (1880) and Meisenheimer (1899), Holmes (1909) and Wierzejski (1905) described it as arising from the mesoderm. In the Stylomatophora, the observations of Meisenheimer (1898, 1899) seems to show clearly that its origin is ectodermal, but according to more recent studies it appears to be only the part near the epidermis which derives from the ectoderm, while the main inner portion is mesodermal (Fernando, 1939; Carida, 1939). With respect to the Pelecypod protonephridium, Meisenheimer (1901) described it as differentiating from the ectoderm but MacBride (1914) claims that it is probably of mesodermal origin. In the Amphineura, the marine Prosobranchia and Opisthobranchia and the Scaphopoda, no protonephridium appears. Instead in the marine Prosobranchia and Opisthobranchia, at each side of the foregut posterior to the velum, a pair of special organs consisting of several cells or one exceptionally large cell protrude from the body wall (Fig. 11.13). This organ is observed in the larvae

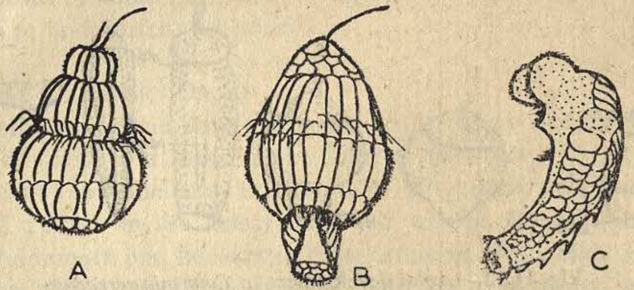

Fig. 11.13. Larvae and young adult of *Nematomeria banyulensis*.
A—Larva at 36 hours after hatching.
B—Larva at 100 hours after hatching.
C—Young worm just after metamorphosis (7 days).

of many of the gastropods which do not have protonephridia, and is considered to be derived from the ectoderm. It does not have an excretory tubule, but performs this function by receiving waste material from the free cells in the body cavity (Portman, 1930).

In addition to the above, in some gastropods, the so-called nuchal cells differentiate in the neck region. These organs are believed to have an excretory function (Wierzejski, 1905, Bloch, 1938), they are also called the cephalic kidneys. Whether these are of ectodermal origin or mesodermal origin is again doubtful. In the prosobranch *Fiona*, an anal kidney differentiates from the ectoderm above the anus in the posterior part of the trochophore larva and the right side of the veliger.

HEAD VESICLE

The part of the larva anterior to the ciliary ring of the velum is called the head vesicle. In this region the apical organ develops as well as the ganglia and other nerves, the eyes, and other sense organs. In the Gastropoda particularly the pulmonate Stylommatophora, it becomes extremely prominent. The head vesicle is formed chiefly by the cells of the lq, but in some cases the 2q cells may be added to these. The apical plate occupies the anteriormost part. The apical plate is usually formed by the four apical cells la^{111}—ld^{111}, although it is said that in *Planorbis* and *Physa* two or three other cells of unknown origin also take part in this formation (Holmes, 1900, Wierzejski, 1905). A large number of cilia are produced on the apical plate. However, according to Casteel (1904) *Fiona* fails to form a distinct apical plate and no cilia appears. Apical cilia are similarly not seen in *Crassostrea*.

The lateral ectoderm of the apical region proliferates and gives rise to the rudiments of a pair of brain ganglia. In the pulmonates, to the left and right of the apical plate cerebral plates are formed and surrounded by apical cells which are said to be derivatives of lq^1 in *Planorbis*. The proliferation of these cerebral plates produces the brain ganglia. It therefore, shows, that the brain ganglia are of ectodermal origin, but Wierzejski (1905) states that mesoderm cells also take part in their formation. But according to Raven (1952) some of the cells which are produced internally by the proliferation of the ectoderm develop into ganglia, while others become mesenchyme cells, corresponding exactly to the mesectoderm of vertebrate embryos.

In Pteropods such as *Vermetes*, *Helia* and *Limax* the rudiments of the brain ganglia arise, not from a simple proliferation of ectoderm cells, but from an ectodermal invagination which appears as tubular tissue.

Among the Amphineura the rudiment of the central nervous system can be recognised as a thread like tissue formed internally as the result of the proliferation of the rosette cells (Hammarsten and Runnstran, 1925).

According to Meisenheimer, in *Dreissensia* the rudiment of the cerebral ganglia appears as a bilobate cerebral pit, from the bottom of this depression are separated off a pair of nerve cell clumps which become the ganglia and the commissure which connects them.

DIGESTIVE SYSTEM

The rudiment of the digestive tract is formed by the invagination of the 4q (except 4d), the 5q and the macromeres (5A—5D). As the result of the unequal proliferation of the body wall cells, the blastopore which was first in the posterior part of the body moves in a ventral direction becoming very small or entirely closed, and in its place a stomodaeum is newly formed by an ectodermal invagination. This makes a connection with the archenteron (midgut) and differentiates into the foregut (oesophagus). In *Lymnaea* some of the archenteron cells absorb the albumen of the egg case and become very large albumen cells (Raven, 1952). The midgut differentiate into the stomach, intestine and digestive diverticula (also called as the midgut glands or liver). The intestine is first recognised as a row of cells which do not form a tube (as in *Fiona*) or as an invagination of archenteron which is tubular from the start (Crepidula). The entoderm cells (entomeres) derived from the 4d blastomere take part in its formation.

The anus opens later at the place where the intestine comes into contact with the ectoderm. At this time in the genera, *Limax* and in *Dreissensia* a very slight ectodermal invagination called as the proctodaeum can be recognized (Meisenheimer, 1898, 1901). According to Wierzejski (1905) the hindgut arises from the primary mesoderm in *Physa* and in *Lymnaea* the hindgut is said to be formed by the growth of the midgut epidermis (Rable, 1879, Fol, 1880; Bloch, 1938 and Raven, 1952).

From the right and left sides of the anterior part of the midgut the digestive glands or liver take their origin. These organs arise as a pair of foliated structures with a lumen. The exact position of these organs is at the junction of oesophagus and midgut. The rudiment of the midgut gland usually appears before the re-opening of the blastopore, but the development of this gland is greatly reported in embryos having a large amount of yolk. In the genus *Buccinum*, there are special cells called as the 'nurse cells'. The developing embryo obtains the energy from these cells. In this case the development of the midgut gland is postponed until the veliger stage.

In the case of pelecypods and plankton eating gastropods there is a 'crystalline' style. The style sac which secretes this differentiates fairly early in the veliger stage from the posterior part of the stomach as a blind tube.

MESODERMAL BANDS

The 4d blastomere sinks inside the blastula and divide. The two resulting cells take a bilaterally symmetrical position and give rise to the mesoderm. These two cells are in contact with each other at the median plane (ME^1,ME^2). In the initial stages these two cells lie on the dorsal side at the posterior end of the embryo. But when the blastopore shifts in a ventro-anterior direction, the two cells are carried to the ventral posterior region. The cells which are derived from them unite to form bands. These are called the mesodermal bands; since the cells which gave rise to them lie at one end and produce them, these are called telo-mesoblasts.

The mode of formation of ento-mesoderm is strikingly similar to that of the annelida, but the difference in the case of Mollusca is the ento-mesoderm does not develop further until the beginning of metamorphosis. Before the formation of a coelomic cavity the mesodermal bands break up into individual cells and become the so-called mesenchyme. The tissues of the larval musculature and other organs are chiefly formed by the ectomesoderm.

Ecto-mesoderm and Larval Muscular Systems

As described above, the mesoderm which has its origin in the ectoderm is called ectomesoderm. These cells are believed to become mesenchyme and form the larval musculature which degenerates at the time of metamorphosis.

The main components of the larval musculature are the retractor muscles of the velum, mantle, digestive tract and foot. These are formed of spindle-shaped cells. In pelecypod veliger larvae, three sets of retractor muscles develop, each of these has an attachment point on the left or right shell near the hinge, while its other end ramifies on the velum, mantle etc. (*Dreissensia*, Meisenheimer, 1901). The action of these muscles draws the soft parts of the larval body inside the shells. In the case of pelecypod veliger stage, an anterior adductor muscle develops at an early stage and this is used for closing the shells. This adductor muscle persists through metamorphosis and changes over into the adult organ.

In the gastropod veliger like *Fiona*, a large retractor muscle appears. This muscle has its starting point in the posterior dorsal part of the body and attaching to the body wall near the digestive tract and oesophagus. One more pair of muscles arise at the left and right sides at the posterior end and each member ramifies to the foot. Besides these there can be seen highly branching muscles which extend from the neck region and the oesophagus to the velum (Casteel, 1904). All these muscles need not be of ectomesodermal origin. Some of them like the bilateral pair of retractor muscles are derived from primary mesoderm cells (ento-mesoderm).

Experimental Evidence Relating to Cell Lineage

Many people have done work on the cell lineage of molluscs. Wilson (1904) worked on the determination of the blastomeres in *Dentalium* and *Patella*. The experiments of Conklin (1902, 1912, 1917) on *Crepidula* are worth mentioning. The early work of Crampton (1896) and the studies of Clement (1952) on *Ilyanassa* are worth mentioning. Berg (1954) has published a study of the same sort on *Mytilus*. From the above mentioned work it is possible to imagine that the various future organs which will form from the several blastomeres are determined in molluscs in the early cleavage stage.

In the development of *Dentalium* a polar lobe is formed in the first three cleavages. If the first polar lobe is removed in the trefoil stage the trochophore developing from the remainder of the egg completely lacks the apical organs belonging to the anterior part of the body and the post-trochal region, the portion of the body posterior to the prototroch fails to develop. If the polar lobe of the second cleavage is removed the posterior part becomes small but in most cases a larva with apical organs is formed. The larva which do not have this polar lobe do not metamorphose, they fail to develop the foot, shell gland, shell, mantle and pedal ganglia. No mouth can be seen and they also lack a coelom and mesoderm cells. If the blastomeres are separated at the 4-cell stage, larvae lacking apical organs and posterior part develop from blastomeres A, B and C while these structures are abnormally large in the larvae formed from isolated D blastomeres. Again, the larvae obtained from isolated 1q micromeres all lack a posterior part, and only in the larva produced from the 1d blastomere are apical cilia formed.

Wilson observed that in the normal development of *Dentalium* the material making up the polar lobe is chiefly shifted into the 2d blastomere (first somatoblast). Probably a part of this is transferred to the 4d (second somatoblast) and 3d blastomeres. He further showed that the posterior part of the trochophore develops from these two somatoblasts. It is therefore possible to anticipate the same result as that seen in the experiments cited above i.e. the posterior part will not develop in blastomeres which do not contain polar lobe material, but it is very interesting that removal of the polar lobe leads to the loss of the apical organs which lie on the opposite side of the larva.

In the case of *Patella* egg a polar lobe is not formed. The two isolated blastomeres of the 2-cell stage develop into larvae possessing apical organs. Also larvae with apical organs have been found to develop from each of the isolated 1q blastomeres. The basic material of the apical organs in *Patella* is believed to be divided equally among the four macromeres at the time of cleavage.

If the blastomeres of *Patella* embryo are isolated at various cleavage stages, each of them follows the developmental course which would have taken as a part of the whole embryo. For instance, a macromere isolated in the 8-cell stage forms a gastrula. The extremity of this gastrula are produced by one or two secondary trochoplasts. At the other end a group of entoderm cells can be seen having delicate cilia characteristic of the normal embryo. The macromeres isolated from the 16-cell stage formed gastrulae having delicate cilia but lacking the secondary trochoblasts. If the primary trochoblasts ($1q^2$ blastomeres) are isolated, they develop into four typical ciliated trochophore cells.

When Conklin (1912) isolated *Crepidula* blastomeres in the 2 and 4-cell stages and observed their later development, he found that the cleavage of each blastomere is the same as it would have been in the nomal embryo. When the D blastomere is included, the 4d primordial mesoderm cell is formed, when the D blastomere is lacking the 4d cell does not appear.

Like *Dentalium*, *Ilyanassa* forms a polar lobe at the time of the first three cleavages. Clement (1952) has first removed the first polar lobe at the trefoil stage and followed the subsequent development. The main points in which this differs from the normal are, that the size difference between the D blastomere and the other macromere disappears while the 4d cell, which is normally composed chiefly of clear cytoplasm, contains a large amount of yolk and presents the same appearance as 4a, 4b and 4c, and fails to produce the mesodermal bands. Moreover, in the normal embryo the cleavage of 3D precedes that of the other macromeres but in lobeless embryo this difference is not seen. The 1d blastomere also normally differs from the other 1q macromere in such characteristics as size and time of cleavage but these differences disappear on removal of the polar lobe. Among the molluscan cross tip cells ($2q^{11}$), the $2d^{11}$ cell is largest in normal embryos, but loss of the polar lobe removes this difference also. At the stage corresponding to the veliger, lobeless eggs develop enough to form abnormal partial larvae having a velum but lacking foot-shell and other organs. Muscle tissue can also be recognised but according to Clement this probably arises from ectomesoderm cells. He reports that embryo from which 4D blastomere is removed become small but perfect veligers.

In the preceding section we dealt with the facts concerning the Gastropoda and Pelecypoda as the subject and described the developmental processes from egg to veliger stage in a general way. This section will treat with various matters connected with the special characteristics of molluscan species in a more particular fashion and describe later development from the veliger to the adult form.

Development of the Marine Snail *Ilyanassa*

The eggs of *Ilyanassa* are particularly interesting for embryological studies because of two facts: (1) During the first cleavage the egg forms a large cytoplasmic protrusion at the vegetal pole known as polar lobe. This polar lobe is very influential in morphogenesis. Since the lobe can be easily detached it has permitted a variety of types of analysis both of the properties of the isolated lobe and of the alteration in properties of the lobeless egg as compared with the normal. In fact, most of the experimental work on *Ilyanassa* has been directed toward the role of the lobe cytoplasm in developmental processes, either at the biochemical or structural level. (2) The eggs can be obtained in abundance throughout the year from snails maintained in small salt water aquaria in inland laboratories. The cultural requirments are simple both for eggs, embryos and adults. The period required for development of a well formed veliger larva is about one week at room temperature and hence sufficiently short to conduct variety of experiments.

The taxonomic designation currently used for the species *Ilyanassa* is *Nassarias obsoletes* (Say). *Nassa* and other generic names have been used in the past. Since *Ilyanassa* is the name generally used by embryologists for *N. obsoletes*, it will be employed in the present account in reference to this species.

Reference will be made occasionally to another marine prosobranch gastropod, *Crepidula*. Generally speaking, *Ilyanassa* is the more favourable form for experimental work on problems of embryonic development. The monumental descriptive works of Conklin (1897, 1902) on the development and cytology of *Crepidula* provide a wealth of detailed information which is not available for *Ilyanassa* and which may be of interest in connection with experimental work on the latter form. *I. obsoleta* is commonly found on mud flats in shallow water along much of the Atlantic coast of the United States, and may often be seen exposed in large numbers at low tide.

The breeding season normally begins with warm weather in the spring and lasts on into the summer. The snails can be kept in aquaria with flowing sea water. For steady egg production the snails should be fed regularly, preferably at the end of each day. The sexes are separate in *Ilyanassa,* fertilization is internal and the eggs are deposited in capsules which in the laboratory are attached to the walls or floor of the aquarium. Each capsule usually contains from 20-200 eggs at approximately the same stage of development. Females in good condition may deposit a row of several capsules. Freshly laid eggs will usually not extrude the first polar body and at room temperature the first cleavage will not take place for about three hours.

The eggs of *Ilyanassa,* once removed from the capsule and jelly mass in which they are normally enclosed, appear perfectly naked. The upper part of the egg is occupied by a protoplasmic cap which is largely free of yolk, and in which the nucleus or division figure is to be found. The remainder of the egg is occupied by yolk granules of widely varying diameter. The protoplasmic cap is rich in both mitochondria and lipid droplets whereas these constituents are relatively sparse in the more yolky region. The polar lobe when it first appears contains much yolk.

With centrifugal force of around 2000 gr. the egg contents are readily stratified into three zones: (1) a centripetal oil cap, (2) a rather granular 'clear' zone containing the nucleus and (3) the centrifugal yolky zone. It is possible to achieve a further separation into five zones: (1) the oil cap, (2) a hyaline zone which contains the nucleus and which appears homogeneous and structureless in the living egg, (3) a mitochondrial band, (4) a second clear zone which contains a few small yolk granules and appears in fixed preparations to be different in nature from the upper clear zone, and (5) the yolk zone.

The fine structure of the polar lobe of *Ilyanassa* at the trefoil stage was described by Crowell (1964). The most prominent inclusions of the lobe were the yolk particles. Elements of endoplasmic reticulum, double membrane vesicle, ribonucloprotein particles and mitochondria were found among them. Yolk particles were found to consist of a central homogeneous core, an outer granular zone and a limiting membrane. The double membrane vesicles of unknown composition and significance were found in the polar lobe at first cleavage or before the trefoil stage of first cleavage in the vegetal region of the egg but not in the animal region. Mitochondria were less abundant in the polar lobe than in other regions. The cortical zone of the polar lobe was found to be narrower than the corresponding zone of the animal region of the egg, and its cytoplasm was dense. Few, short microvillii were found on the polar lobe surface. Whereas on the animal region numerous long microvillii were present. The cortex of the egg and polar lobe contain many RNP particles.

Development from Deposition of the Egg to Veliger Larva

Both Margon (1933) and Cather (1963) have reported observations on the *Ilyanassa* egg between the time of laying and first cleavage and the latter author has constructed a detailed schedule of sequence of nuclear events and associated changes of the egg during the period of meiosis and early cleavage.

Although the freshly laid egg may sometimes show an intact germinal vesicle, usually the first polar spindle is forming deep in the cytoplasm of the animal third of the egg, where a sperm has entered. In other cases the spindle is already at the surface.

The eggs within a capsule will usually be at approximately the same stage.

As the first meiotic spindle of the fertilized egg moves to the surface at the animal pole, a brief and rather inconspicuous bulge at the vegetal pole comprises the first polar lobe. The first polar body appears and within a few minutes the egg is again spherical and remains so for about 15 minutes. The second polar lobe then appears, just prior to the metaphase stage of the second meiotic division. It becomes large and conspicuous and persists altogether for about 50 minutes. The second polar body is given off approximately 20 minutes after the appearance of the second polar lobe, and about this time the lobe shows irregularities of outline not seen at other stages. The irregularities disappear and the resorption of the lobe is followed by another spherical period.

Following the second meiotic division the sperm nucleus, previously small and dense, enlarges and assumes a characteristic interphase condition. It then migrates below the egg pronucleus which has entered the prophase condition. The polar lobe makes its appearance during late prophase while the asters are enlarging and before the pronuclear membranes have disappeared. The time lapse between the disappearance of the second polar lobe and the appearance of the third is about 55 min. The third polar lobe (Fig. 11.14) enlarges to a prominent condition during metaphase. During anaphase the animal pole flattens and the egg elongates in the plane of the cleavage spindle. As the cleavage furrow is formed, the lobe is further constricted from the egg until the trefoil stage is reached (Fig. 11.14). At trefoil the egg consists of three spheres of roughly the same size, two blastomeres at the polar lobe. A fine cytoplasmic filament extends from the upper end of the polar lobe to the

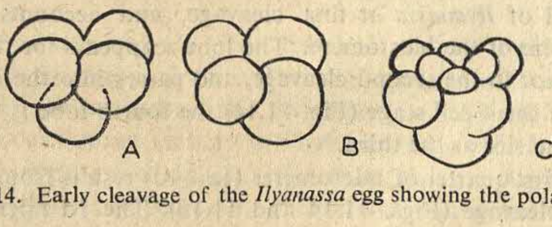

Fig. 11.14. Early cleavage of the *Ilyanassa* egg showing the polar lobe.

notch at the bottom of the two blastomeres. At this time it is not evident with which of the blastomeres the lobe fuses eventually. The size of the third polar lobe varies in different lots of eggs from about one quarter to one third the volume of the whole egg. It is easy to distinguish the polar lobe from the blastomere. The polar lobe is very yolky in appearance. The blastomeres on the other hand have a lighter upper polar area, where the nucleus and the large yolk-free cytoplasmic cap occurs. The trefoil stage occurs about three hours after the time of laying and the beginning of meiosis. The polar lobe in the beginning is attached to one of the blastomeres. Slowly the connection broadens and eventually the polar lobe is incorporated into the blastomere. In this way an unequal first cleavage is achieved, the larger blastomere which has received the lobe is known as CD, and the smaller as AB (Fig. 11.14).

In the genus *Crepidula* the first cleavage appears to be equal although Conklin (1902) has described a small cytoplasmic lobe, probably homologus with the polar lobe of *Ilyanassa*, which is attached and reabsorbed into one of the first two blastomere.

Development from First Cleavage to Larva

The early cleavage of *I. obsolete*, through the initial subdivisions of the mesentoblast cell, was described in detail by Clement (1952). No comprehensive account of later development has been published. As mentioned, the polar lobe passes into the CD cell of *Ilyanassa* at first cleavage, and accounts for the larger size of the blastomere. The lobe reappears for the fourth time prior to the second cleavage, and passes into the D blastomere of the 4-cell stage (Fig. 11.14) the fourth lobe is of about the same size as the third.

The first quartet of micromeres (1a—1d) results from a dexiotropic cleavage (Figs. 11.14 and 11.15). The 1d micromere is noticeably smaller than the other three, eventhough 1D is the largest of the four macromeres. The second quartet is formed by a leotropic division and all cells of this group (2a—2d) are of similar size and appearance (Fig. 11.15). After the appearance of the second quartet, the cells of the first quartet subdivide to produce the turret cells ($1a^2$—$1d^2$), which are much smaller than their sister cells ($1a^1$—$1d^1$) lying near the animal pole. The smaller 1d micromeres subdivides later than the other three (Fig. 11.15).

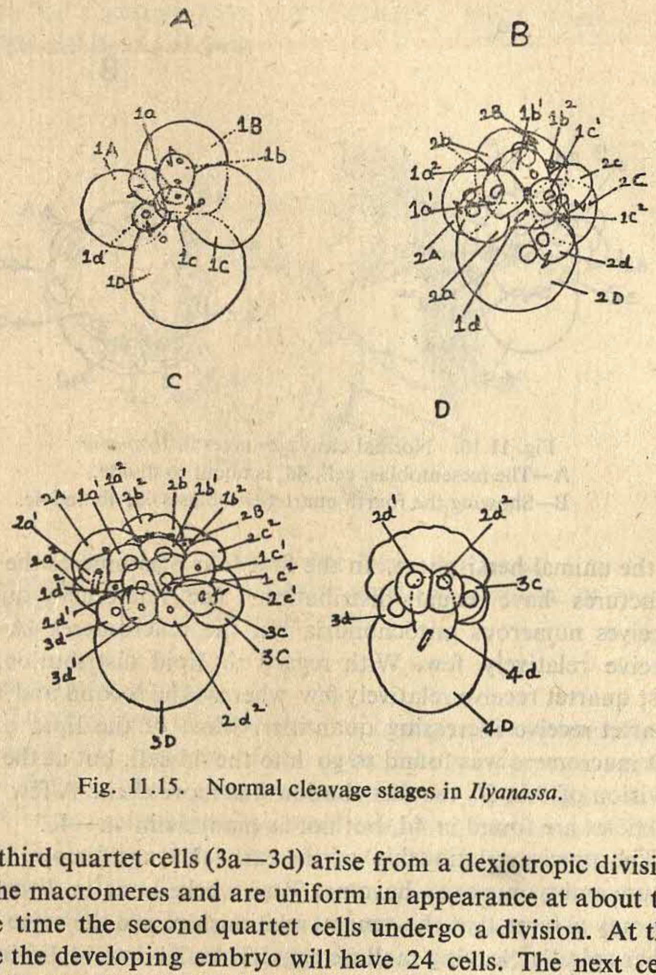

Fig. 11.15. Normal cleavage stages in *Ilyanassa*.

The third quartet cells (3a—3d) arise from a dexiotropic division of the macromeres and are uniform in appearance at about the same time the second quartet cells undergo a division. At this stage the developing embryo will have 24 cells. The next cells to divide are the apical cells $1a^1$—$1c^1$. After that the mesentoblast cell, 4d makes its appearance. The interval between first cleavage and the appearance of 4d is about 6-6½ hours at 25-26°C. The entoblastic derivatives of the fourth quartet (4a—4e) appear about 3 hours after the formation of 4d. Meanwhile 4d will have divided equally in the plane of bilateral symmetry of the embryo, and the resulting cells will have begun the subdivisions which eventually segregate some entoblastic cells and form the primary mesoblast bands (Fig. 11.16). In the uncleaved egg the lipids and mitochondria are found in a broad cytoplasmic cap

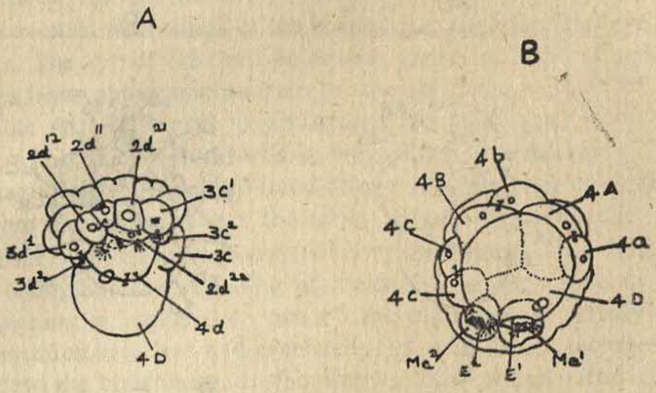

Fig. 11.16. Normal cleavage stages in *Ilyanassa*.
A—The mesentoblast cell, 4d, is about to divide.
B—Showing the fourth quartet entoblasts 4a, 4b and 4c.

in the animal hemisphere. In the first four blastomeres the two structures have equal distribution. The micromere quartet receives numerous mitochondria but the macromeres 4A—4D receive relatively few. With regard to lipid distribution, the first quartet receive relatively few whereas the second and third quartet receive increasing quantities. Most of the lipid of the 3D macromere was found to go into the 4d cell, but at the subdivision of 3A, 3C the distribution was more even. A few yolk particles are found in 4d, but not as many as in 4a—4c.

The precise relationship of the early cleavage furrows to the future embryonic axes, have not been worked out in *Ilyanassa*, we may assume that the general relationships are the same as in other spirally cleaving mollusc eggs. Thus 2d and 4d lie in the median plane and 4d is at the posterior end. If viewed from the animal pole of the cleaving egg, the A quadrant would lie to the left of the median plane and the C quadrant to the right.

Gastrulation is by epiboly. By the time of hatching of the veliger, the yolk has been largely or entirely absorbed and various organs can be seen clearly in the living larva. The prominent parts of the larva are the paired velar lobes and eyes, the foot with a pair of statocysts and an operculum, the larval shell, the digestive tract and a beating heart. Some of the details are shown in Fig. 11.17.

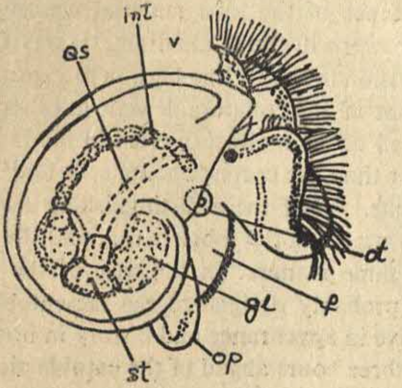

Fig. 11.17. Normal veliger larva of *Ilyanassa*.
os—oesophagus; f—foot; gl—digestive gland; int—intestine; op—operculum; ot—statocyst; st—stomach; v—velum.

EMBRYOLOGICAL STUDIES

In the early days of experimental biology, Crampton (1896) discovered that removal of the polar lobe of *Ilyanassa* at the trefoil stage of first cleavage prevents the appearance of a characteristic mesoblastic pole cell and the formation of the mesoblast bands. After detachment of the polar lobe, the blastomeres of the 2 and 4-cell stages were of equal size. Successive quartets of micromeres were produced, but the fourth derivative of the D quadrant (4d) lacked the characteristics of the typical mesoblast cell and instead resembled the 4a, 4b and 4c entoblastic cells of the other quadrants. The lobeless embryos lived long enough to develop cilia and swim around but they showed little differentiation under the culture conditions employed.

A careful, step-by-step comparison of cleavage in the lobeless and the normal embryo revealed a further difference between the two as early as the first quartet of micromeres (Clement 1952). In the normal egg the first micromere derivative of the D quadrant (1d) is smaller than the other three, whereas in the lobeless egg all first quartet micromeres are of the same size. The presence of the lobe material in the D blastomere in some unknown manner causes this cell to produce a smaller micromere than would otherwise be the case. In a number of other ways the presence of the lobe material was shown to modify the cleavage pattern in the D quadrant. It was found, that the rate of subdivision in the 1d line lags, in the normal egg, as compared with that of the comparable cells in other quadrants and that the tip cell of the molluscan cross in the D quadrant ($2d^{11}$) is much larger than the corresponding cells ($2a^{11}$, $2b^{11}$, $2c^{11}$) of other quadrants. All of these distinguishing features of the D quadrants disappear in the lobeless egg, and the four quadrants cleave in the same manner. In the case of the fourth quartet, the 4d cell (probably designated the mesentoblast cell) is not only distinctive in appearance and history in normal egg, it also arises about three hours ahead of the entoblastic fourth quartet cells (4a, 4b, 4c) of the other quadrants. In the lobeless embryo all of the fourth quartet cells are yolky and similar in appearance and they also appear simultaneously. It is thus evident that the presence of the lobe material confers special characteristics on the 4d cell, and causes the cleavage which produces it to occur precociously by about three hours.

The mesentoblast cell (4d) normally marks the posterior pole of the future embryo since a typical mesentoblast cell is not produced if the polar lobe is removed at first cleavage, and the lobeless embryo does not show any clear indication of an antero-posterior axis in other respects, it appears that the lobe is associated either as cause or effect, with the establishment of the posterior pole.

Under suitable conditions, the lobeless embryo differentiates certain larval tissues and parts but fails to develop a great many others. The parts which typically fail to develop may be called as lobe-dependant structures. These include eyes, foot, statocysts, operculum, external shell, heart and intestine. The structures which develop are velar cilia, small cilia, an everted stomodaeal structure, some differentiation of entodermal tissue, pigment, and active muscle fibers. In general appearance, the lobeless larva bears little resemblance to the normal veliger (Fig. 11.17). The velar tissue commonly occurs in several masses and lacks the organization it assumes in the normal veliger.

The development of isolated blastomeres of the 2- and 4-cell stage demonstrates that the morphogenetic influence of the polar lobe is allocated to the CD blastomere at first cleavage, and to the D blastomere at the second cleavage. Isolated CD blastomeres develop into larvae which may show parts such as eyes, foot and external shell (Fig. 11.18), whereas AB blastomeres

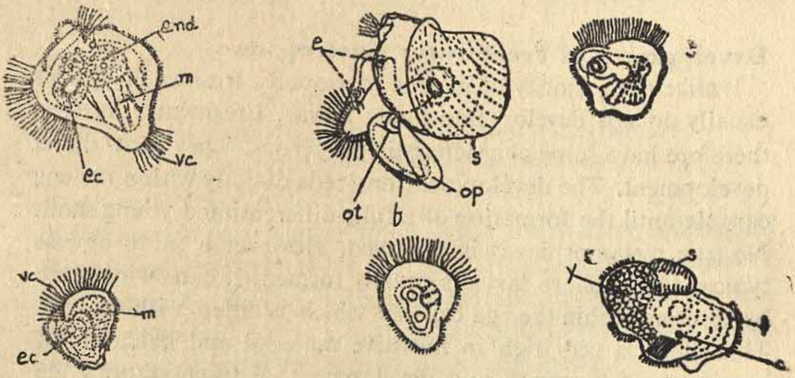

Fig. 11.18. Partial larvae of *Ilyanassa* from isolated blastomeres. e—eye; ec—enteric cavity; end—entoderm; f—foot; m—muscle; op—operculum; ot—statocyst; s—shell; vc—velar cilia; y—yolk.
A—Larva from AB half.
B—Larva from CD half.
C—Larva from A blastomere.
D—Larva from B blastomere.
E—Larva from C blastomere.
F—Larva from D blastomere.

give partial larvae lacking the lobe-dependent features (Fig. 11.18). Only the isolated D blastomeres of the 4-cell stage differentiates lobe-dependent features. Although D blastomeres in general tend to develop poorly, rare cases may differentiate an eye and a small external shell (Fig. 11.18). All classes of isolated blastomeres from the 4-cell stage may differentiate velar cilia, pigment and entoderm. The general appearance of the partial larvae from A, B and C quarters is simliar (Fig. 11.18).

The development of isolated one quarter blastomeres thus indicates that the morphogenetic influence of the polar lobe accompanies the physical bulk of the structure into the D macromere at the second cleavage.

It is during the period of micromere formation that the morphogenetic influence of polar lobe region is exerted (Clement 1962). If the large 4D macromere containing the bulk of the yolky material originally contained in the polar lobe, is removed from the embryo following the stage of formation of the 4d micromere, a small but well formed veliger develops. By this time the 4D macromere is of nutritive importance only.

Development of Freshwater Gastropoda

Unlike the majority of marine gastropods, freshwater species usually do not develop free living larvae. Freshwater species, therefore have some characteristic properties. They show direct development. The development proceeds directly within the egg capsule until the formation of a fully differentiated young snail. No true metamorphosis is observed. However a more or less typical trochophore larva is usually formed. It can swim freely by rotating within the egg capsule which is filled with a fluid. This fluid is very rich in nutritive material and hence is of importance for growth and development. A trochophore stage is quite normally formed, but the veliger stage in most freshwater gastropoda is not typically formed. The vela which are so prominent in the veliger of the marine gastropoda is not formed.

Development of *Limnaea stagnalis*

Eggs of *L. stagnalis* are laid in the form of egg batches which contain from 20 to 80 egg capsules. The pulmonate snails to which *L. stagnalis* belongs are usually hermaphrodite but sterile themselves. During copulation spermatozoa are exchanged between both partners. Insemination occurs within the oviduct. During the formation of an egg batch insemination of the individual eggs occurs one after the other in the sequence in which they are included in the egg mass.

Three different egg membranes can be distinguished (Fig. 11.19). (1) The primary or vitelline membrane is excreted by the cell cortex of the oocyte. In *Limnaea* it is very thin and delicate, nearly invisible in the light microscope but definitely seen with the aid of the electron microscope. (2) The secondary or chorionic membrane is formed by follicle cells. It envelops a

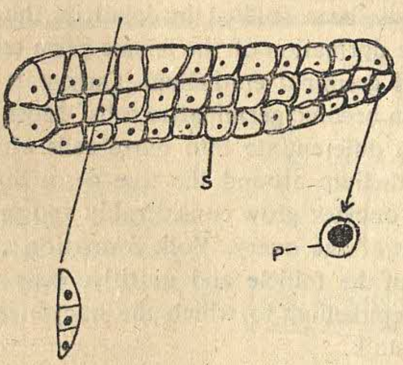

Fig. 11.19. Egg batches of *Bithynea tentaculata*. p, s—primary and secondary egg membrane.

rather large capsular space which is filled with a nutritive liquid. The egg cell swims freely in this fluid during the later stages of development. (3) The tertiary membrane or jelly coat is formed by the cells of the oviduct. It is a jelly skin which enwraps the free egg capsules to form an united egg batch.

Two types of eggs have been distinguished (1) The regulation eggs, and (2) mosaic eggs. The principal differences between these types is the stage at which the differentiation capacities of the egg or embryonic areas are irreversibly determined. In eggs of the regulative type this determination occurs at a comparatively late stage. In the eggs of the mosaic type determinations occur at rather early embryonic stages. In embryos of the regulation type the differentiation of a morphogenetic area very often depends upon its situation within the whole embryo and is influenced or even directed by surrounding tissues. In contrast to this, in embryos of the mosaic type differentiation not only occurs rather early but very often is also irreversible.

For a long time the embryos of the gastropoda were considered to be typical examples of the mosaic type of differentiation. But, today it is generally accepted that there are no sharp but only gradual difference between the two types of differentiation.

OOGENESIS

Oogenesis has been studied in detail in the pulmonate *L. stagnalis*. The dormation of the female germ cells occurs within a so-called germinal epithelium. Only part of the cells of this epithelium become germinative cells. Most of the cells are determined to differentiate into nurse cells which form a type of follicle epithelium around the true germ line cells. During oogenesis the oocytes grow considerably and protrude into the central cavity of the ovary. Yolk formation takes place by a cooperation of the follicle and nutritive cells with the cells of the germinal epithelium to which the oocyte is still connected by a slender stalk.

In the stage of the immature egg, the cells are clearly radially symmertically organized. The egg has an animal vegetative axis which is indicated by the eccentrical location of the egg nucleus with its special clear perinuclear cytoplasm. There is some evidence that the vegetal pole of the egg coincides with the point at which the growing oocyte had been attached to the germinal epithelium.

Before the beginning of the first cleavage division a drastic change in the distribution of the components of the egg cytoplasm occurs. Before the egg enters into the first cleavage division, the more or less homogeneously distributed egg components are dislocated and a specific pattern is developed. This process has been called 'ooplasmic segregation'. The most conspicuous result of ooplasmic segregation is the formation of polar plasma which in different species are found either at the animal or at the vegetal pole or at both.

CLEAVAGE

The eggs of the freshwater gastropoda are cleaved according to the well known spiral pattern which is generally observed in molluscs. The first two cleavages divide the egg in a meridional direction into four blastomeres which form the quadrants A, B, C and D (Fig. 11.20). This is followed by four cleavage divisions in which the cleavage furrows are oriented in the equatorial plane. The divisions are strongly unequal. The smaller blastomeres are oriented towards the animal pole. Thus four quarters of micromeres are formed. The spindles of these cleavage divisions are not oriented exactly perpendicular to those of the first two divisions but are shifted in a lateral direction. Because of this, if they are viewed from the animal pole, the micromeres are not located exactly above their corresponding macromeres, but are displaced laterally.

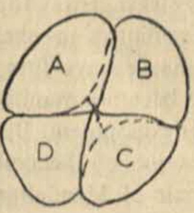

Fig. 11.20. Inverted spiral cleavage in dextrally coiled animals.

In consecutive cleavage divisions spindles become oriented alternately in a clock-wise and an anti-clockwise fashion. Thus dexiotropic divisions are followed by laeotropic divisions.

In this way, all blastomeres become arranged in a strongly regular pattern.

In freshwater gastropoda, as a rule the first two cleavage furrows occur meridionally. The blastomeres of the 4-cell stage are often of equal size. In spite of this, the differentiation capacities of the four quadrants are not the same. The D-quadrant has specific capacities to differentiate into mesoderm and its descendants which the other quadrants do not possess.

In *L. stagnalis* no polar lobe is observed and the four qurdrants appear to be of equal size. However, in *B. tentaculata* a polar lobe is formed during the first cleavage division.

But, when compared with marine species here the polar lobe is so small that it is hardly observable. It fuses with the D-quadrant, but because of the smallness of the lobe the D-cell cannot be distinguished by its size from the three other blastomeres. Even though no experiments have been conducted on the morphogenetic role of this small polar lobe, it is well known that mesoderm originates also in this species from the descendants of the D-quadrant.

Limnaea, like most of the freshwater gastropoda, possess a shell which is dextrally coiled. Sometimes individuals with a sinistrally coiled shell are found. The dextral or sinistral coiling of shell is genetic. There is a very important correlation between the directions of shell coiling and the orientation of the cleavage furrows. According to the normal spiral type of cleavage, dexiotropic cleavages are followed by laeotropic ones and vice versa. In eggs of a normal specimen of *Limnaea*, the third, fifth, seventh etc. cleavage steps are dexiotropic. Correspondingly the fourth, sixth, etc. divisions are laeotropic. However in individuals with sinistrally coiled shells inversed cleavage was found (Fig. 11.20). Thus, genes controlling the coiling of the shell also control in some way the orientation of the cleavage furrows and therefore their phenotypic expression becomes manifest during the very early stages of embryonic development. By crossing experiments it was found that the direction of the coiling of the shell is determined by one single pair of Mendelian alleles. The normal allele causing a dextral shell is dominant over the mutant 'sinistral shell'.

GASTRULATION

Gastrulation takes place by two different processes: (1) Invagination of entodermal material into the blastocoel, and (2) Epibolic growth of the marginal regions of the ectoderm. These two types of gastrulation depend upon the type of blastula, size of the blastocoel and the amount of yolk present in the entoderm cells. For example, in the embryos of *L. stagnalis* invagination of the entoderm is more pronounced, whereas in embryos of *B. tentaculata* both processes participate at about equal rates to gastrulation.

These two processes mostly take place independently of each other. In *B. tentaculata* gastrulation starts with the formation of a comparatively shallow pit at the vegetative pole. This arises by changes of the shape of the ectoderm cells in this region. The external surface of these cells is reduced at the same time their inner parts increase in width. The invagination is completed as the marginal ectoderm grows epibolically over the entoderm. Such a growth of one cellular layer over the other require certain properties in the cell surface of the two germinal regions. In half embryos, which develop from isolated blastomeres, gastrulation starts normally with a shallow invagination of the ectoderm. During the following phase only a part of the ectoderm is capable of growing epibolically over the entoderm. In some regions of the half embryo, this movement appears to be inhibited. Despite this, growth of the ectoderm seems to be normal. Thus, a peculiar asymmetric gastrula is formed. Because one side of the ectoderm is not able to glide over the entoderm as would be normal, it has to advance in the direction of the animal pole. As a result a vesicle is formed. This vesicle enlarges quickly and is filled with liquid. Because the liquid develops some pressure, the continuation of the invagination of the entoderm is not only inhibited, but on the contrary the early shallow invagination of the archenteron is finally everted. In this way, half embryos of *B. tentaculata* develop into exogastrulated embryos.

Unlike the observations just described in embryos of *L. stagnalis* epibolic growth of the ectoderm does not seem to play a similarly important role during gastrulation.

EMBRYOGENESIS

In gastropoda, the gastrula develops into a trochophore larva. This stage is also found among the freshwater species which does not have free living larva. It rotates with the aid of its trochus in the nutritive liquid of the egg capsule.

The older trochophore larvae exhibit the typical tripartite organization of molluscs in head, foot and visceral mass. The shell gland of the trochophore is developed from the gastrula in the following way. The late gastrula stage typically shows an increased growth of the postrochal regions of the dorsal ectoderm. In this way the blastopore is displaced to the ventral side of the embryo. Afterwards also in the ventral region of the ectoderm growth commences and leads to the formation of an ectodermal evagination in the post-trochal region of the ventral side. This evagination differentiate into the foot analogue. At the same stage a part of the ventral ectoderm sinks into the blastocoel and forms the stomodaeum. The archenteron separates into the anterior and posterior lobes of the midgut gland (anterior and posterior diverticula of the digestive gland) and into the midgut which develops the proctodaeum. Thus the basic plan of the future young snail has been completed.

Little is known about the mechanisms of the differentiation processes during this part of the development. The differentiation in the pretrochal region of the *Limnaea* embryo starts with an easily recognizable and characteristic pattern of blastomeres. This whole ectodermal region is formed by cells which are derived from the first three quartets of micromeres. The cell divisions of the micromeres and their descendants are regulated in such a way that small and large cells are formed which are arranged in a characteristic pattern. Directly at the animal pole of the embryo a small area of large ectodermal cells is found. They form the so-called apical plate. Just beneath the apical plate on both sides two regions composed of small ectodermal cells can be observed. These two areas are the 'cephalic plates'. They are surrounded again by big-celled ectoderm which during further development gives rise to the head vesicle. Finally this tissue is bordered by the cells of the trochal ring which are especially large. Similar topographical relationships of ectoderm cells of different size classes are found in exogastrulated embryos. This demonstrates that the development of the characteristic cellular pattern in the ectoderm of the head region is independent from the underlying entodermal material.

ORGANOGENESIS

Our knowledge about the process of organogenesis is rather poor. However, some experimental results have been received about the formation of the shell gland and the stomodaeum.

THE SHELL GLAND

The shell gland appears for the first time in young trochophore larvae at the dorsal side of the post-trochal ectoderm. At first the primordium of the shell gland consists of a thickening of ectodermal cells (Fig. 11.21). Later, in the centre of the thickened region an invagination of a part of the enlarged cells takes place (Fig. 11.21). During further development the shell gland is displaced to the left side of the embryo. Simultaneously the shell gland enlarges more and more (Fig. 11.21). The central part of the invagination rise again to the surface of the embryo (Fig. 11.21). Thus the shell gland changes from the form of a shallow trough to a circular groove which in still later stages becomes the mantle fold. The groove surrounds an area which is called the shell field. This field secretes the shell primordium in the form of a thin larval shell composed of conchioline. During development the shell groove extends more and more (Fig. 11.21), and the shell (Mantle) field finally covers large parts of the visceral hump.

As far as can be seen shell glands are in all cases are of similar size, specially, there are no size differences in shell glands of whole embryos that have developed from separated blastomeres.

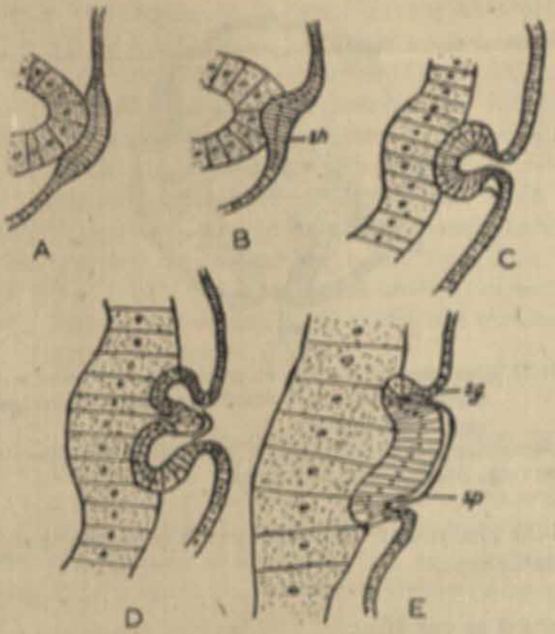

Fig. 11.21. A-D—Developmental stages during the formation of a shell gland.
sh—shell gland; sg—shell groove.

STOMODAEUM

The formation of the stomodaeum is principally different from the development of the shell gland as just described. In normal development a small area of ectodermal cells in the vicinity of the blastopore invaginates into the blastocoel to form the stomodaeum during the transformation of the gastrula into a trochophore larva (Fig. 11.22). An ectodermal tube is formed, the inner end of which remains closed in the beginning stages. Only later it opens into the cavity of the midgut which in the meantime has been developed. The next step in the differentiation of the stomodaeum is its subdivision into pharynx, the radular sac and the oesophagus.

During the early stages in which the funnel-shaped stomodaeum is still closed, its cells are already histologically different from the rest of the ectoderm and can easily be distinguished. Later, differentiation proceeds and the oesophagus, the radular

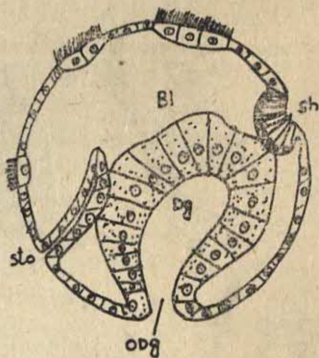

Fig. 11.22. Development of older exogastrulated embryo of *Bithynia tentaculata* after the delayed secondary invagination of a part of the entoderm.

Bl—blastoderm; Dg—digestive gland; Sh—shell gland; Sto—stomodaeum; Odg—opening of digestive gland.

sac and the pharynx are built up by cells of a specific and easily recognizable type.

THE ORGANS OF THE HEAD

There are some observations dealing also with the development of the head organs, such as tentacles, eyes and statocysts.

Amphineura

SOLENOGASTRA OR APLACOPHORA

Since there is very little detailed information concerning the development of these species and many points remain ambiguous, it was not discussed in the previous section. Baba (1938, 1940, 1943, 1951) has published reports on a Japanese member of the Proneomeniidae, *Epimoria verrucosa* (Fig. 11.12). In this species, the egg develops by spiral cleavage, produces apical cilia and an anterior prototroch at about the centre of the embryo, and becomes a trochophore with the blastopore on its ventral side. Later the posterior end of the trochophore elongates and a pedal groove is formed on the ventral side of this region. The tail extremity elongate still further, and a terminal prototroch is formed at its end. At the time of metamorphosis, this tail region, including the prototroch, as well as most of the cells which made up the larva in the trochophore stage, are cast off and the adult worm-like form is assumed.

The larva of *Nematomenia banyulensis* (Fig. 11.13) resembles a trochophore with a conspicuous invagination at the vegetal side, the larval epidermis, which is formed of five rows of cells, is shed in metamorphosis, and the adult stage develops from the portion within the invagination and another part which extends posteriorly from this (Fig. 11.13). At metamorphosis the part which changes over to become the adult body (Fig. 11.13), develops lateral foliate plates and seven small calcareous plates along the back. These accessory structures are eventually exchanged for small calcareous spines.

CHITONS

Loricata: The later development of the chitons has been studied by Heath (1899) and Kowalevsky (1883).

The trochophore larvae of these species do not have protonephridia, but they do form the radula sac, pedal gland and other structures characteristic of molluscs, and pass through a free swimming stage (Fig. 11.23). In general this swimming period is very short, lasting several hours, or at most 2-3 days. At its end the larva begins to creep on the bottom with the foot which has developed on its ventral side during this period (Fig. 11.23). Together with the degeneration of the apical cilia and proto-

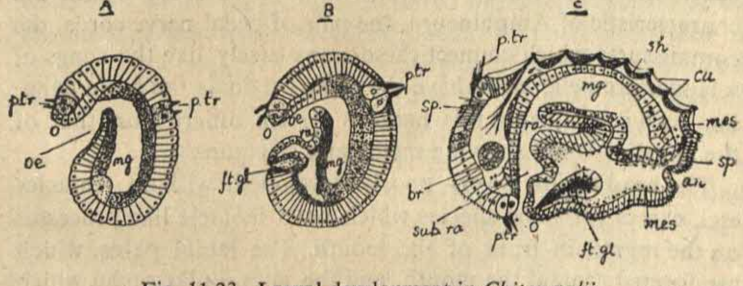

Fig. 11.23. Larval development in *Chiton polii*.
A-B—Sections of trochophore larvae.
C—Section of larva which has assumed bottom living habit.
an—anus; br—cerebral ganglion, cu—chitin; ft. gl—foot gland; mes-mesoderm; mg—midgut; o—mouth, oe—oesophagus, ptr—prototroch, ra—radula sac; sh—secreted calcareous shell; sp—spine; sub-ra: subradular organ.

troch, the posterior part of the body grows and assumes the adult form. A pair of eyes develop as temporary larval organs on the upper part of the foot posterior to the trochophore. These disappear with the completion of the mantle and shell.

One of the major distinguishing characteristics of adult chitions is the possession of a shell consisting of eight overlapping plates, the rudiments of these are also seen in the larvae. By the time the larva changes to a bottom crawling life, a transverse line first appears on its back, followed by six transverse folds which develop at successively posterior levels. A cuticle is secreted over the whole dorsal surface, while calcareous shells are secreted over the parts between the transverse folds. The eighth shell plate is formed considerably later than the others. The shell structure of these animals is complicated, the most conspicuous feature of the shells consisting in the fact that they are pierced at the sides by a number of cytoplasmic processes (the aesthetes) arising from the mantle epithelium. The ends of these processes perform a sensory function at the shell surface.

The brain ganglia arise from four radially arranged rosette cells. The paired longitudinal pleural nerve cords which are characteristic of Amphineura, the pair of pedal nerve cords, the commissures which connect these transversely like the rungs of a ladder are believed to have the same origin as the brain ganglia. As a consequence this nervous system differs from that of the annelids is not showing metameric structure.

The head region is very poorly developed, without tentacles etc., except for some species which have tentacle like processes on the mantle in front of the mouth. The labial palps, which are formed around the mouth, and the subradular organ which is formed in the stomodaeum in front of the radula sac as an invagination, stomodaeal epidermis (Fig. 11.23) are also thought to be sensory organs.

SCAPHOPODA

The development of Scaphopoda has been studied by Lacaze-Duthiers (1851), Kowalevsky, (1983) and Wilson (1904). The trochophore larva is characterized by having three rows of ciliated cells, but in general the mode of development of its various organ systems is closely similar to that of the

Amphineura, Gastropoda and Pelecypoda (Fig. 11.24). The mantle, as in the case of Pelecypoda, develops first as paired

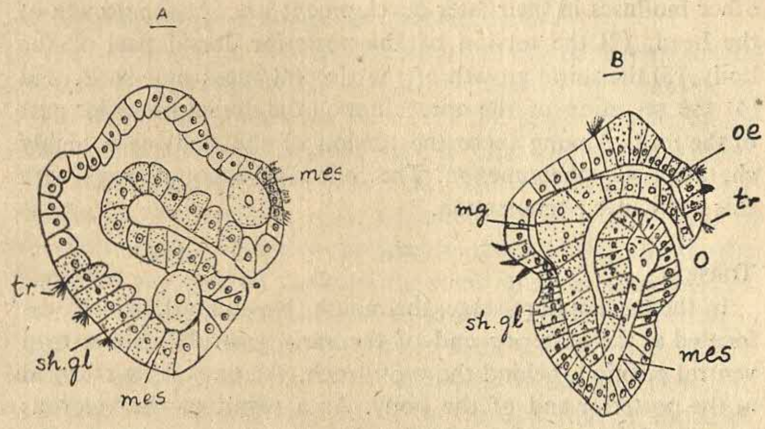

Fig. 11.24. Longitudinal sections of trochophore larvae of *Dentalium*.
A—Larva at 14 hours after fertilization.
B—Larva at 34 hours.
mes—mesoderm; mg—midgut, o—mouth; oe—oesophagus; sh.gh—shell gland; tr—trochal ring.

structures which later fuse ventrally. Because of the shape of the shell, as seen from the dorsal side, at first resembles that of the gastropod trochophore and from the ventral side that of the pelecypod larva. Eventually the two edges fuse ventrally forming a tube. The opening at the posterior end is small, and the shell growth takes place at the anterior end. The foot is well developed, its division into three lobes forming one of the characteristic features of the scaphopod veliger larva (Fig. 11.25).

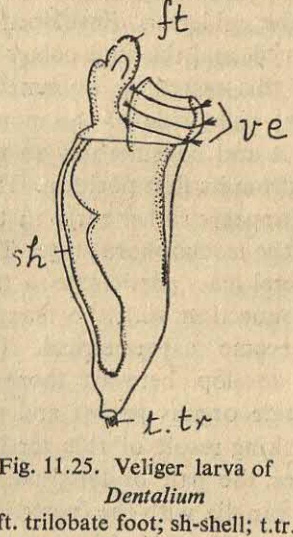

Fig. 11.25. Veliger larva of *Dentalium*
ft. trilobate foot; sh-shell; t.tr. telotrochal ring; ve—velum.

No protonephridia have been observed, but statocysts and pedal glands appear and a radula sac is formed at a later stage.

Later Development of Gastropoda (After veliger larva)

The important points in which the gastropoda differ from other molluscs in their later development are (1) the growth of the head, (2) the torsion of the postesior dorsal part of the body, (3) the spiral growth of the visceral mass and shell, and (4) the secretion of the operculum on the dorsal posterior part of the foot. Among these the torsion of the body is a highly characteristic phenomenon. The following account deals with this particular phenomenon.

TORSION

In the trochophore stage, the mouth (blastopore), which was located at the posterior end of the early gastrula, moves to a ventral position, behind the prototroch. An anus is first formed at the posterior end of the body. As a result of the vigorous growth of the posterior dorsal region, the distance between the mouth and anus, becomes retatively short. In other words, the digestive tract bends markedly forward towards the ventral side. This phenomenon is also seen in the Pelecypoda, where it is called ventral flexion. In the case of the Gastropoda, this growth of the posterior dorsal region is asymmetrical, the left side growing more markedly in most cases. As a result of this, the anus comes to be located at the right side of the mouth. The mantle develops as a fold of the epidermis. Enveloped in this mantle, a mantle cavity is formed, and the anus comes to open into this cavity. As a result of this extremely asymmetrical growth, the anus moves past the right side of the mouth and finally takes a position above it and considerably to the left. It makes a turn of nearly 180° from its first position. This phenomenon is called torsion, and appears rather early in the development, in the latter part of the trochophore stage. The mantle cavity, the shell and the visceral mass participate in this torsion. The organs which have connection with the mantle, like gills, heart and kidney also become asymmetrical. The organs of the right side usually develop between those of the left-side. Sometimes the right side organs persist and the opposite members degenerate. A striking result of this torsion is that the pleuro-parietal connectives, the pair of longitudinal nerves joining the (anterior) pleural ganglia with the (posterior) parietal ganglia cross each other. As a result of this the right nerve is above the oesophagus and the left one below it forming a figure 8. This is known as chiastoneury, and becomes an important characteristic of the Prosobranchia.

In most of the Opisthobranchia, this torsion secondarily undergoes a complete reversal at the time of metamorphosis. The mantle cavity becomes located in the posterior part of the body and the crossed nerves revert to their original condition. This phenomena is called detorsion. However, a gill and auricle develop on one side only. Chiastoneury does not occur in the Pulmonata. This may be due to the fact that the ganglia concened are located close to the head and are therefore not involved in the torsion process, or that the degree of torsion is slight. The asymmetry, already extreme as the result of this torsion, becomes still further accentuated by unequal growth in a different direction from that of the torsion causing the shell and visceral mass to develop in spiral fashion.

SHELL AND OPERCULUM

In the great majority of Gastropoda the shell when viewed from above is dextrally twisted (in a clockwise direction), although a few species show sinistral (anticlockwise direction) twisting, and in a few others both dextral and sinistral types are found. The mantle cavity, anus and genital pore are situated on the left side of the body in the sinistral forms. In the dextral forms they are located in an opposite direction. This dextral or sinistral organization is an inheritable character that is already determined in the unfertilized egg.

The larval shell (shell of veliger stage) is first secreted by the shell gland and later by the mantle. This shell can be easily distinguished by its colour, shape and markings from the shell which is formed after metamorphosis. In some species, even if the adult stage do not have a shell, a larval shell is invariably secreted by the veliger shell gland. At the time of metamorphosis this shell is enveloped in the mantle tissue and disappears.

The operculum is secreted on the dorsal side of the foot as early as the late trochophore stage. The growth of the operculum also takes place in a spiral fashion but in the direction opposite to that of the dorsal shell, so that animals with dextral shells have sinistral opercula. An operculum is always formed in the larva, even in species in which none is found in the adult.

The shell of the adult gastropod is usually composed of an outer layer (periostracum) consisting mainly of conchiolin, a middle layer (ostracum) of which the chief component is calcium carbonate, and an inner layer (hypostracum), this three layered structure closely resembles that of the pelecypod shell.

NERVOUS SYSTEM

When compared with other molluscan classes, the gastropods have strikingly well developed nervous system, possess many ganglia in the larval stage. These ganglia are the cerebral, pleural, pedal, parietal, visceral and buccal ganglia. These develop from ectodermal thickenings which differentiate inward.

The central nervous system like the apical plate arises from the cells of 1q. The buccal ganglia are formed by proliferation of the cells making up the walls of the stomodaeum. The various sense organs develop along with the development of the nervous system. In the veliger stage the head is already provided with antenna and eyes, while a pair of statocysts are well formed at the base of the foot.

Coelom, Pericardium, Kidney, Heart and Blood Vessels

The mesodermal bands arise from a pair of teloblasts. The cells making up these bands separate and become mesenchyme. Then they reaggregate into paired cell masses. Eventually a coelomic cavity appears in each of these, the two cavities develop in close contact with each other, and finally the intervening septum disappears, giving rise to a single large cavity.

This is the pericardium. The part of the pericardium which originates in the right coelomic cavity is better developed than its counterpart on the left side.

The cells on the left and right sides of the ventral part of the pericardium proliferate, producing thickenings which eventually evaginate. These are the rudiments of the kidneys. The kidneys are paired to begin with, but the one on the right side develops well, while the one on the left side degenerates, and the animal thus comes to have a single kidney. The evagination mentioned above develops into the kidney by forming with its walls a great number of folds and pouches. The mouth of the evagination becomes reduced in size but a narrow connection between the pericardium and the kidney is maintained.

The urinary duct connecting the kidney with the exterior, as well as the opening of this duct, are formed by an invagination of the wall of the mantle cavity opposite the kidney. Such invaginations actually occur on both the left and right sides, the one on the right side developing and establishing contact with the right kidney while the one on the left side degenerates together with the left kidney rudiment.

The cells of the pericardial wall above the kidney proliferate, giving rise to a thickening which sinks inward to form a groove. This groove is eventually closed over, except for its two ends, forming a tube, which is suspended in the pericardium. This constitutes the heart rudiment, and its two ends communicate with the primary body cavity. This tubular heart is constricted at its middle into two parts; the anterior part becomes the rudiments of the ventricle and the aorta.

The blood vessels appear as sinus like spaces in the mesenchyme tissue even before the development of the heart. The sinuses are surrounded by flattened cells. Their cavities become more restricted as development proceeds and contribute to the formation of the blood vessels.

REPRODUCTIVE ORGANS

Observations made on the prosobranchs indicate that the gonads, like the kidneys develop by proliferation of the cells forming the wall of the pericardium. The reproductive organs of the prosobranchia are considered to be of mesodermal origin.

Pelecypoda

The pelecypod trochophore differs from the larvae of the other molluscan classes in failing to form a radular sac. As in the gastropods, the shell is first secreted by the shell gland on the dorsal side of the embryo. Eventually this differentiates into two, left and right valves which make contact in a straight line in the region of the shell gland. By the time the shell valves develop sufficiently to cover the soft parts of the body, they exhibit a D-shape. The straight part of the D-shaped larva is the hinge line, teeth differentiate on the two opposing shell valves at this line. The larvae having shells of this shape are called "D-Shaped larvae". A larva having such a shape can be found in practically all pelecypod species in the early veliger stage except in the ones where the young are reared in a brood pouch.

After this, the shell shows more marked growth in the dorsoventral direction than antero-posteriorly and becomes rounded. Eventually the umbo region becomes prominent. The velum is well developed at this time extending beyond the shell and moving vigorously during swimming and feeding activity. At times the larva may draw the velum inside the shell and close the valves abruptly sinking to the bottom. In the central part of the velum apical cilia are usually developed.

Muscles extending from the dorsal body wall (region of the hinge line) attach to the velum and digestive tract and by their contraction the soft parts can be gathered completely inside the shell. An interior adductor muscle also develops near the anterior part of the shell for the purpose of closing the valves. The development of the posterior adductor muscle occurs much later.

The opening of the anus occurs early in the D-shaped larval stage. With this the larval digestive tract becomes functional, and feeding activity begins. The paired rudiments of the digestive diverticula appear at this stage and the left member of the pair develops rapidly. The intestine is a straight tube at the beginning, becomes looped as the development progresses. The stomach also develops and at its posterior end, a large blind sac, the crystalline style sac appears.

The larval nervous system is extremely simple when compared with the adult. It consists of cerebral ganglia below the apical plate, rudiments of pedal ganglia in the region between mouth and anus, where the foot will later develop. Visceral ganglia develop near the pedal ganglia and the parietal ganglia close to the cerebral. A pair of statocysts appear in the vicinity of the pedal ganglia. Below the velum a pair of black pigmented eyespots or pigmented spots appear.

A protonephridium of ectodermal origin develops near the tips of each of the pair of long, narrow mesodermal bands as one of the larval organs. The adult kidney is also formed adjacent to these.

The foot forms on the ventral side of the body between the mouth and the anus. The foot becomes well developed at the time when the veliger larva changes from a free-swimming to a bottom dwelling mode of life. The larva sometimes crawls about with its foot which is covered with slender cilia, and sometimes swims with its velum.

The ectoderm, at the base of the foot invaginates, giving rise to the pedal gland or byssus gland, when the larva takes up a benthic mode of life, it clings to the substratum with mucus or byssus threads secreted by this gland. In common mussels this gland is well developed in the adult form also. When the adult does not have such a gland, it degenerates in a latter developmental stage. The cement gland of the oysters is similar in nature to this gland.

The mantle appears first in the trochophore stage as an expansion of the ectoderm in the posterior part of the larva, and subsequently develops as lateral folds extending to its anterior end. The shell, which is secreted by the mantle, also grows along with the development of the mantle. The ctenidium appears in the late veliger stage as a row of papillae, and develops rapidly after metamorphosis. The inner pair of ctenidial lobes appear earlier than the outer pair.

In the late veliger stage, spindle-shaped mesenchyme cells aggregate at a point anterior to the anus and form the posterior adductor muscle. When the veliger changes to a bottom dwelling mode of life, around the larval shell, an adult shell of different nature is formed. As the veliger assumes the bottom dwelling habit, most of its tissues become disassociated and are discarded or absorbed (Fig. 11.26).

The mesodermal bands are poorly developed in the early stages. The pericardium and the accessory organs have a mesodermal origin. The cells of the anterior dorsal parts of the left and right mesodermal bands forms two vesicles or coelomic cavities. These constitute the rudiment of the pericardium. The cavities on the two sides of the intestine increase in size, eventually coming together and fuse (Fig. 11.27). Between this cavity and the intestine the ventricle is formed and the inner wall of the cavity becomes the ventricular wall. A little bit earlier than this, invaginations appear in the two cavities and

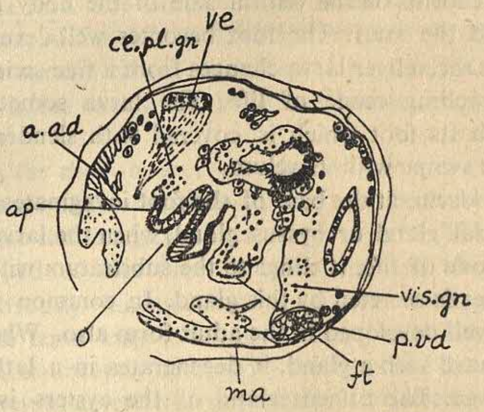

Fig. 11.26. Young oyster, *Ostrea edulis* 24 hours after fixation. Foot and velum have degenerated.
a.ad—anterior adductor; ap—apical plate; ce.pl.gn.—cerebropleural ganglion; ft—foot; ma—mantle; o—mouth; p.ad—posterior adductor; ve—velum; vis.gn.—visceral ganglion.

these form the rudiments of the auricles which later make connections with the ventricles (Fig. 11.27).

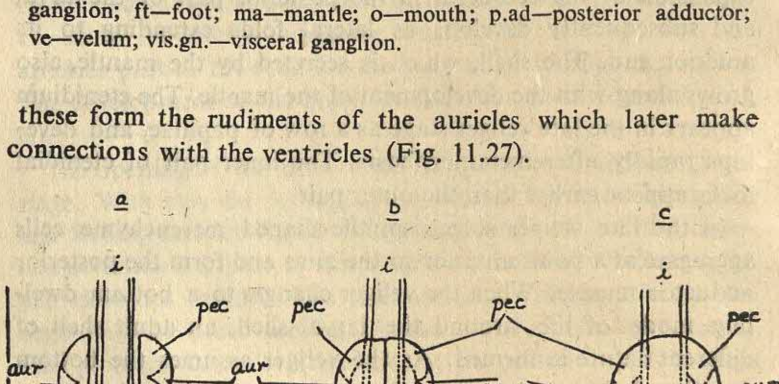

Fig. 11.27. Development of pericardium and heart in *Cyclas cornea*.
aur—auricle rudiment; i—intestine; pec—pericardium; ven—ventricle; x—line of fusion of right and left pericardial cavities.

The mesodermal cells adjacent to the pericardium form a filiform tissue, which develops a lumen and eventually develops into the kidney. This kidney soon opens into the pericardium while the other end extends towards the body wall and eventually forms an opening to the outside. The pericardial rudiment can be seen in the veliger stage, but the pulsation of the heart cannot be detected until after the bottom dwelling habit has been assumed.

The reproductive organs maintain an intimate connection with the pericardium, heart and kidney and are believed to be of mesodermal origin. If an independent genital pore is formed, it is always close to the urinary duct. In many cases a common opening serves both for urinary and genital ducts. In some cases the reproductive cells are released into the kidney itself.

This larva does not have a velum, foot and mouth. The eggs of freshwater species belonging to the family Unionidae develop for a certain period in brood pouches formed by the water-tubes of the maternal gill laminae. When they leave the brood pouch, they are in a larval stage, which is called the *Glochidium* larva. These larva are extraordinary without any velum, foot and mouth. Without going through a free-swimming period, these larvae become parasitic on the fins or other scaleless parts or on the gills of various fishes. On the host body, these larvae metamorphose and become adults. The adults leave the host and take up a bottom-dwelling mode of life. The glochidium larva which parasitize on fishes, the valves of the shell are provided with a pair of curved hooks on their ventral edges. They develop a single, large adductor muscle in the centre of the body. When a fish comes close to the glochidium, the larva use this muscle to clap its valves together. It moves upward, secures a firm hold on the fish body and becomes parasitic. The cells on the inner part of the mantle develop large vacuoles and become columnar. These feed on and absorb blood and tissue cells of the host fish. In the process of metamorphosis, these special larval organs degenerate and are replaced by adult organs.

Cephalopoda

The cephalopoda are the most highly evolved of the classes which make up the phylum Mollusca. The cephalopods are usually marine, heterosexual. The eggs are usually large and heavily laden with yolk. The species which inhabit the coastal areas deposit their eggs in egg masses which are generally non-buoyant whereas the species living in the open sea often lay buoyant eggs.

EARLY DEVELOPMENT

Among the cephalopoda, the spermatozoa does not leave the body as separate cells but as spermatophores enclosed in tubular sheaths (Fig. 11.28). After the spermatozoa have been

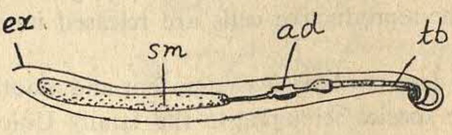

Fig. 11.28. Spermatophore of *Sepiella maindroni*.
ad—adhesive body; ex—external sheath; sm—mass of spermatozoa; tb—emission duct.

formed in the testis, they pass through a long, slender twisted sperm duct into the vesicula seminalis. Then they are arranged into small lots which are enclosed into spermatophores. These are temporarily collected in bundles in a spermatophore sac (or Needham's sac) where they are stored until copulation takes place. At this time the males use its hectocotylized arms to implant the spermatophores into the thin mantle cavity of the female or on the buccal membrane surrounding the mouth.

The eggs of *Sepia* are attached to the sea weeds separately by thin gelatinous white coats and are often covered with sand grains. About 45 minutes after fertilization, the first division begins with the appearance of a cleavage furrow passing through the animal pole (Fig. 11.29). This gradually elongates so that it includes more than two thirds of the egg diameter at one hour after fertilization. About an hour later the second cleavage furrow begins to appear at the same point as the first but perpendicular to it (Fig. 11.29). At about three hours after fetilization the third division sets in, perpendicular to the second cleavage furrow (Fig. 11.29). The points where these furrows begin are near the first cleavage plane, but whereas they run parallel to it on one side of the egg, they diverse from it by about 45° on the other (Fig. 11.29). The fourth division is completed about four hours after fertilization and results in the formation of two centrally located micromeres (Fig. 11.29). After

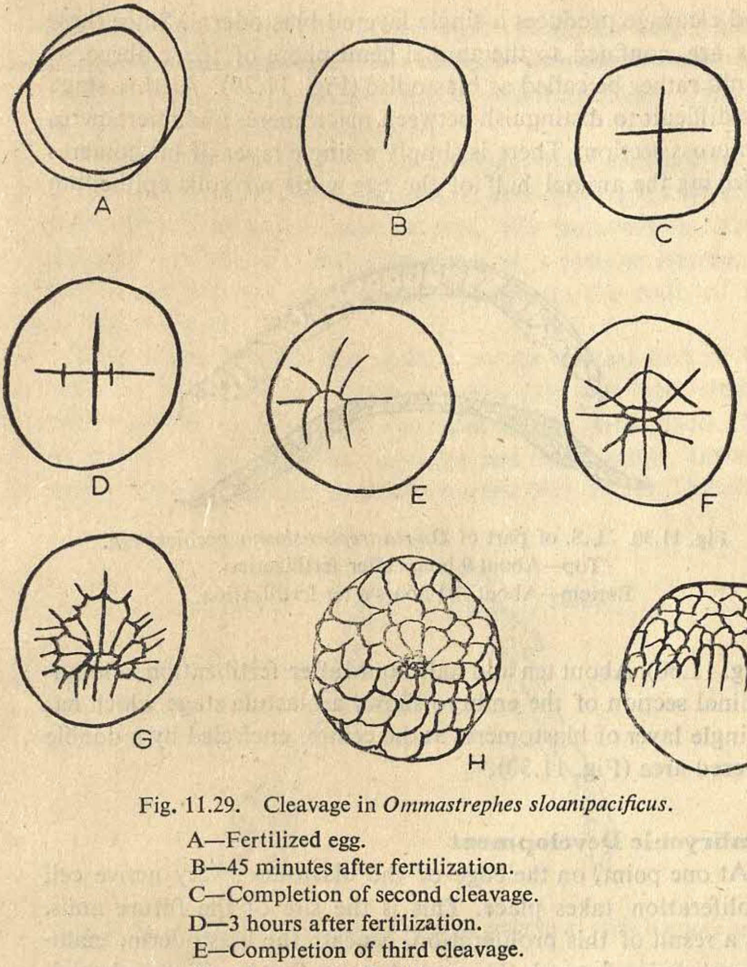

Fig. 11.29. Cleavage in *Ommastrephes sloanipacificus.*

A—Fertilized egg.
B—45 minutes after fertilization.
C—Completion of second cleavage.
D—3 hours after fertilization.
E—Completion of third cleavage.
F—4 hours after fertilization.
G—Fifth cleavage.
H—About 6½ hours after fertilization.
I—About 8½ hours after fertilization.

this, the rate of cleavage begins to show local differences and the marked increase in the number of furrows makes it difficult to follow the segmentation process by simply observing the surface of the embryo. By six and half hours after fertilization, 32 to 64 cells can be seen, and the number of central micromeres is eight (Fig. 11.29). During the next two hours, continued cleavage produces a single layered blastoderm. Since these cells are confined to the animal hemisphere of the embryo, it should rather be called as blastodisc (Fig. 11.29). At this stage it is difficult to distinguish between macromeres and micromeres in a cross section. There is simply a single layer of blastomeres overlying the animal half of the egg with no yolk epithelium

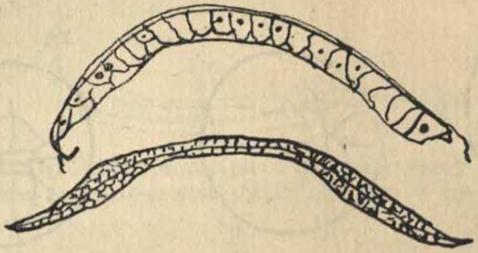

Fig. 11.30. L.S. of part of *Ommastrephes-sloanic pacificus* egg.
Top—About 9 hours after fertilization.
Bottom—About 10½ hours after fertilization.

(Fig. 11.30). About ten and half hours after fertilization, a longitudinal section of the embryo shows a blastula stage which has a single layer of blastomeres at the centre, encircled by a double layered area (Fig. 11.30).

Embryonic Development

At one point, on the edge of the blastodisc, very active cell proliferation takes place. This is the site of the future anus. As a result of this proliferation, beneath the blastoderm, endoderm is being formed. The endoderm at first is crescent-shaped, which soon enlarges and becomes circular. Thus the embryo acquires two layers, the ectoderm of the blastodisc overlying the newly formed endoderm. The ectodermal cells of the anal side proliferate laterally, and extend toward the anterior in the space between the ecto and endoderm layers, to give rise to the mesoderm. The endoderm thickens along the middle line to form a vesicle, which is the archenteron. From the archenteron a stomach, intestine and a bilobed liver are differentiated. The anal invagination forms near the vegetal pole of the blastodisc, and the stomodaeal invagination opposite it. The stomodaeal invagination gives off the buccal mass, radula sac and salivary glands and connects with the already formed stomach. The proctodaeum is formed later and is connected later with the intestine by a shallow invagination.

To the right and left of the intestine, the mesoderm forms vesicles, called as the body cavities. The kidney arises from the inner walls of these coelomic cavities. The pericardium, which eventually gives rise to the heart arises in a similar manner to that of the kidneys. The gonads arise from the walls of the coelomic cavities.

In the meantime, on the surface, at the central part of the blastodisc, the ectoderm bulges to give rise to a heart-shaped protuberance, which is the rudiment of the shell gland. The periphery of this rudiment thickens and then bends inward, finally drawing together to produce a sac (Fig. 11.31). This shell sac, within which the shell will be formed does not correspond to the shell gland seen in the embryos of other molluscs. Around the periphery of the shell sac, the rudiment of the mantle appears as another protuberance, and the ectoderm at the lateral and posterior edges of the blastodisc gives rise to outwardly directed processes, the arm rudiments (Fig. 11.32). As these arms elongate, they also move along the sides of the body towards the mouth, and their bases unite and finally surround it.

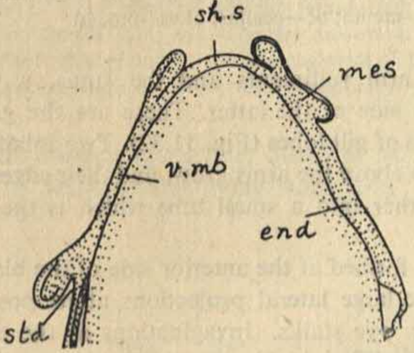

Fig. 11.31. L.S. of part of *Loligo vulgaris* embryo at early blastoderm stage.

end—endoderm; mes—mesoderm, sh.s—shell sac; std—stomodaeum, v.mb.—vitelline membrane.

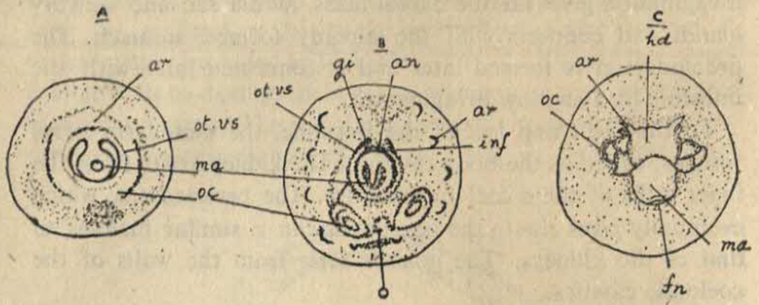

Fig. 11.32. Three successive stages of blastoderm development in *Sepiella maindroni*.
A—Embryo at 11 days after first cleavage.
B—More advanced stage.
C—21 days after first cleavage.
an—anus; ar—arm; fn—fin rudiment; gi—gill; hd—head; inf—funnel; ma—mantle; o—mouth; oc—ocellus; ot.vs—otocyst.

Between the arm rudiments and the anus, a protuberance arises on each side of the latter. These are the gill rudiments and form folds of gill lobes (Fig. 11.32). Two lobate projections which develop above the arms grow, and their edges turn inward and join together into a small tube which is the funnel (Fig. 11.33).

The head is formed at the anterior side of the blastodisc (Fig. 11.32) and two large lateral projections at its posterior corner represent the eye stalks. Invaginations at the ends of these become the eyes (Figs. 11.32 and 11.33). The brain, optic, visceral and pedal ganglia, all originate separately as streak like areas of the ectoderm which gradually sink below the surface as they become differentiated (Fig. 11.33). Two ectodermal masses at the sides of the optic nerves are the white bodies, which are believed to be vestiges of the lateral lobes of the brain ganglia. The cranial cartilage which is characteristic of the cephalopods is formed by a modification of the connective tissue, derived from the mesoderm near the bases of the arms.

These developmental changes occur at the expense of the yolk, which is gradually reduced in amount. The visceral sac expands rapidly and soon becomes larger than the head. As the embryo acquires a shape practically identical with that of the

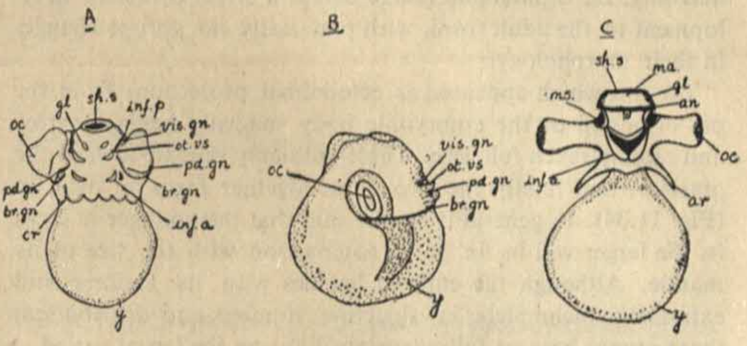

Fig. 11.33. Embryos of *Loligo vulgaris*.
A—Embryo viewed from posterior.
B—Embryo viewed from side.
C—More advanced stage.

an—anus; ar—arm rudiment; br-gn—rudiment of cerebral ganglia; gi—gill rudiment; inf.a. rudiment of anterior funnel fold; inf. p—rudiment of posterior funnel fold; ma—mantle; ms—retractor muscle of funnel; oc—ocellus: ot.vs—otocyst; pd.gn—rudiment of pedal ganglion; sh.s—shell sac; vis.gn—rudiment of viscereal ganglion; y—yolk.

adult, it secretes a hatching enzyme, dissolves its chorion, and swims forth to begin its free-living stage (Fig. 11.34). After

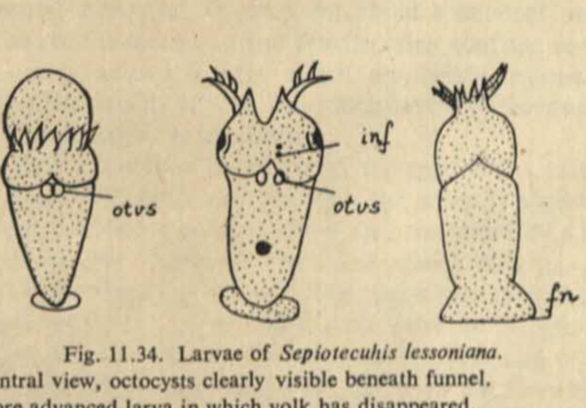

Fig. 11.34. Larvae of *Sepiotecuhis lessoniana*.
A—Ventral view, octocysts clearly visible beneath funnel.
B—More advanced larva in which yolk has disappeared.
C—Dorsal views of same larva in which fin is still limited to posterior end.

fn—fin; inf—funnel; ot.vs—otocyst.

hatching, the cephalopod larvae pursue a direct course of development to the adult form, with practically no abrupt changes in their morphology.

The fins which appeared as ectodermal projections from the posterior end of the embryonic body suddenly begin to grow and rapidly reach full size. These gradually spread toward the anterior, and finally the two sides together form an oval fin (Fig. 11.34). In general it can be said that the younger a larva is, the larger will its fin be in comparison with the size of its mantle. Although the embryo hatches with its suckers still extremely incomplete in structure, number and distribution, these organs become fully developed during the larval period.

The eyes of cephalopods have a structure amazingly similar to that of the vertebrate eye, and their function is believed to be highly developed. Although the statocysts, which like the eyes are formed as ectodermal invaginations at a very early stage in the development of the blastoderm, are at first only simple sacs, they occupy a prominent position at the centre of the embryo and are clearly visible even from the outside.

12. *Echinodermata*

The phylum Echinodermata includes the classes Echinoidea (sea urchins, sand dollars), Ophiuroidea (brittle stars), Asteroidea (starfishes), Holothuroidea (sea cucumbers) and Crinoidea (sea lillies). This group is considered to be so highly evolved and is placed close to the vertebrates. However, its body structure at first glance seems similar to radially organized coelenterates and other animals. Once the embryology of these animals is known, it is apparent that their radial organization is secondarily acquired as the result of their derivation from sessile ancestors.

Echinoidea

EARLY DEVELOPMENT

Echinoids have been famous objects for embryological and experimental biological research for about a hundred years. This is due to the fact that a ripe female often contains several million easily accessible eggs which are highly transparent because of the paucity of yolk, and which develop rather quickly and synchronously after fertilization.

From their maturation in the ovary, the eggs of sea urchins have a polar differentiation. The eggs are normally shed after the second maturation division. They are surrounded by a jelly coat of about 20μ. Normally the entire sperm, with the tail, enters the cytoplasm of the egg. The sperm then migrates towards the egg nucleus or is transported there by cytoplasmic streamings and the sperm nucleus swells by loosening of the densely packed chromatin. The resulting zygote (fertilized egg), nucleus then moves back to the egg centre. After the penetration of the sperm into the mature egg, the outer membrane of the double plasmalemma becomes elevated to form a fertilization membrane. The fertilization membrane is smooth, transparent, and about 0.1 μm thick. Fertilized eggs can be easily distinguished from unfertilized ones by the elevated fertilization membrane.

The polar axis is included in the first cleavage plane, which divides the egg into two blastomeres. The second cleavage is equal, and also includes the egg axis, so that the resulting four blastomeres are evenly grouped around it. The central space between the blastomeres is the beginning of the blastocoel. The third cleavage is again equal, but the plane of cleavage is at right angles to the egg axis, dividing the four blastomeres into upper and lower halves and thus giving rise to two layers of four blastomeres each. Since these eight blastomeres are all of the same size, it is still impossible to distinguish between the animal and vegetal poles. The fourth cleavage is highly characteristic. The four cells of the upper layer divide equally and vertically, forming eight blastomeres of the same size arranged in a ring, while the lower four cells divides horizontally into two layers. Their cleavage is extremely unequal, so that the upper layer consists of large cells and the lower of four very small ones. In the 16-cell stage, then the blastomeres are arranged in three layers, an upper layer containing eight medium-sized mesomeres, a middle layer containing four large mesomeres and a lower layer composed of four small micromeres. At the next cleavage, the ring of eight mesomeres divides into upper and lower layers. All these 16 cells thus formed are destined to become ectoderm, but for convenience in tracing their later distribution, each layer has been given a particular designation; the upper layer is known as an_1 and the lower as an_2. The macromeres divide vertically, forming a single ring of eight cells, and the micromeres cleave horizontally into two layers. The embryo as a whole thus consists of a total of 32 cells arranged in five layers.

The stage from 32 cells to 64 cells is known as the morula stage. During this phase, the cells grow successively smaller with each doubling of their number and also the embryo becomes gradually smoother.

BLASTOCOEL

After it begins to divide, the embryo increases in size at each cleavage. This enlargement is not due to the increase in the amount of protoplasm but due to the expansion of the blastocoel (Fig. 9.2 h Kume). The synchrony of cleavage continues beyond the seventh cleavage, and can be followed until the time of hatching at the tenth cleavage. The size of the blastocoel, however, stops increasing at the seventh cleavage, and remains constant until the embryo hatches. Cilia develop on the surface of the blastomeres even before hatching. Late blastula stage rotates within the fertilization membrane. At the thicker blastoderm epithelium of the animal pole, the cilia are longer, denser and stiff; this is known as the ciliary tuft. The blastula produces a hatching enzyme capable of dissolving the fertilization membrane. The time required from fertilization to hatching is 5 hours at about 30°C.

Blastula: The blastula immediately after hatching is practically spherical. The cell layer at the anterior end thickens and changes into a sensory element provided with a tuft of only immobile cilia. This structure, which obviously enables the embryo to sense the presence of obstacles that it meets, is called an apical organ.

Primary mesenchyme cells: About an hour and a half after hatching, a change can be observed in the blastular wall at the vegetal pole side. Some of the blastomeres, which until this time have been arranged in an orderly row begin to slip out of line into the blastocoel. These cells, after their separation from the blastular wall, are known as primary mesenchyme cells, and their immediate function is to form the spicules. Although a large number of cells have escaped into the blastocoel, no gap is formed in between the cells, because as soon as these cells slip out of the lineup, the surrounding cells draw together and replace them. In order to avoid the formation of gaps, the cell wall in general has to spread out toward the vegetal side. As a result of this shift in position, the posterior wall of the embryo after this time is composed of cells descended from the veg_2 blastomeres. At this stage, the embryo is rather pointed at the anterior side, while the posterior side formed by the veg_2 cells is quite flat.

The process of gastrulation begins about ten hours after fertilization depending upon the temperature. Simultaneous with this activity, however, is the formation of spicules by the primary mesenchyme cells.

SPICULE FORMATION

The primary mesenchyme cells after becoming disengaged from the blastular wall move sideways and gather in two places in contact with the inner surface of the vegetal body wall. These two places are never exactly on opposite sides of the blastular periphery—actually, their radii form an angle of about 140° at the centre. No one has yet discovered why the mesenchyme cells choose such unlikely positions, but at any rate the side subtended by the 140° angle is the ventral side of the embryo, and the 220° angle subtends its dorsal side. With the choice of these two places, the embryo at one time acquires not only bilaterality but also dorsoventrality. In the middle of each of these accumulations of mesenchyme, a certain number of the cells become arranged in a triangular formation and eventually a triradiate spicule appears at their midpoint. The spicules have the appearance of being formed in the space between the mesenchyme cells, but it is believed that these cells use their pseudopodia to form a matrix, and the spicule is actually laid down within this matrix sac. There is a general belief that a mutual influence between the physiological properties of the animal and vegetal poles plays a highly significant role in sea urchin development. When the primary mesenchyme cells are in contact with an, spicule formation will be impaired by an excess of the animal pole characteristics possessed by the an_1 cells, and when they are in contact with veg_2, by the too strongly vegetal character of the veg_2 cells. The successful skeleton formation occurring in collaboration with veg_1 must be due to the appropriate polarity characteristic of the veg_1 cells. Emphasizing the animal characters and diminishing the vegetal character is called animalization while the opposite process, increasing the vegetal characters at the expense of the animal is called vegetalization.

GASTRULATION

The first morphological change associated with gastrulation is the closer aggregation and growth in height of the veg_2 cells as distinguished from those of the veg_1 group, which remains constantly flat. The second change is the gradual sinking of the veg_2 group as a whole into the blastocoel. Since the aggregate has the shape of a circular plate, it is called the endodermal plate. As a consequence of this change, a longitudinal section of the primitive gut (archenteron) at the beginning of invagination resembles a low, flat topped table.

Soon after the endodermal plate begins to sink into the blastocoel, pseudopodia appear on the cells situated in the centre of the plate. These are the secondary mesenchyme cells. However, these secondary mesenchyme cells, unlike those of the primary mesenchyme cells which extend only along the inner surface of the blastular wall, traverse the blastocoel and reach the opposite ectodermal wall. With the formation of the pseudopodia, the flatness of the endodermal plate is lost, and the archenteron changes to a tall, slender cylinder. Although the pseudopodia at first extend radially, they are eventually directed toward the animal pole and following the lead of these pseudopodia, the archenteric tip establishes connection with the animal pole. In forms where the blastocoel is too large for the pseudopodia of a single cell, some of the cells leave for archenteric tip precociously and form bridges across the space to make connection with the animal pole.

SWIMMING LARVA

Pyramid stage

The pyramid stage follows the gastrula. The body axis of the gastrula tilts to one side to give rise to this stage. Because of this tilting, the larva assumes a pyramid shape with the aboral side as the apex and the oral side as the base. This is an important period during which the establishment of the body organization and the growth of the spicular skeleton, the formation of the stomodaeum, the tissue differentiation of the digestive system and the separation of the coelom are taking place.

SKELETON FORMATION

If the triradiate spicule mentioned above is superimposed, two of the three branches of the spicule are seen to fall on the plane of the page while the last branch stands perpendicular to the page and is directed toward the median plane of the larval body.

Each triradiate spicule soon acquires a new branch which is in line with the body rod, but directed in the opposite sense. These are the post-oral rods. If the body rods and the post-oral rods elongated on the same line, the natural consequences will be that their ends will push out the body wall, forming projections. The two body rods come together on the aboral side and form a single common projection, while the two post-oral rods diverging on the anal side, give rise to two projections. These two projections are two post-oral arms. The third branch of the original spicules is seen to extend up along the page and bend orally. This is the antero-lateral rod. After the larva reaches a fairly advanced stage, the antero-lateral rods of the two sides separately extend to form a pair of antero-lateral arms.

Although the formation of these arms has so far been described as though it resulted simply from an outpushing caused by the elongation of the rods, the real situation seems to be somewhat more complicated. For instance, if the development of the post-oral rod is suppressed, the ectoderm destined to form the arm proliferated and gives rise to wrinkles or vesicles in spite of the absence of the rod. However, these vesicles fall away as long as the rod formation is completely suppressed. In other words, the skeleton and the ectoderm cooperate to produce the arm.

It has already been stated that triradiate spicules are formed within two matrices lying among the primary mesenchyme cells. Consequently, as the spicule develops into a skeleton, the direction of its elongation, its shape and its branching are all governed by the cast of the matrix; the shape of this structure, in turn, is determined by the pseudopodia of the primary mesenchyme cells.

TISSUE DIFFERENTIATION

As the archenteron tilts to one side a shallow depression appears in the ectodermal wall on that side. This is the stomodaeum. The blind end of the archenteron, as the result of this tilting, comes into contact with the depression, and an opening is made through the two layers to form the mouth of the larva. A band of strong cilia differentiates on the body surface surrounding the larval mouth. The stomodaeum derived from an blastomeres of an early stage, was for some time believed to be induced by the tip of the archenteron, because partial larvae consisting of an_1 cells fail to form this structure. After the formation of the mouth, the rest of the digestive tract ceases to be a simple cylinder. The front point, continuous with the mouth, becomes narrow and slender as the oesophagus. The middle part expands to become the stomach and the rear part changes into the slender intestine, thus establishing the so-called tripartite digestive tract. The blastopore of the gastrula stage remains unchanged and serves as the anus.

To form the coelom, the mass of secondary mesenchyme cells which lies between the archenteric tip and the animal pole is divided into two groups when the archenteric tip unites with the stomodaeum. These give rise to a pair of small coelomic vesicles on the sides of the oesophagus, the rudiments of future coelom. Before this appearance of the coelom, the free space within the larval body is provided by the blastocoel.

When the larva of this stage swim, they show a queer rolling motion quite different from the regular spiralling of the blastula and gastrula. This is because the pyramid stage represents a transition between the gastrula and the pluteus stages during which the axis of swimming motion is changing.

Pluteus Stage: External Appearance

The pluteus stage is a continuation of the pyramid stage. Due to the elongation of the skeletal rods, the aboral end is situated to a more acute point and the postoral and antero-lateral arms extend directly outward from the sides of the stomodaeum. When the larva reaches this shape, the body axis deviates from that of the gastrula by nearly 90°, and the larva begins to swim with the pointed aboral end down and the four arms up. The larva at this stage has the shape of a pyramid with four legs, or perhaps of an inverted stepladder. Johannes Muller, named this larva, the 'pluteus', because of the similarity which he saw to an easel.

The pluteus stage is reached within few days following fertilization. More than a month is required before metamorphosis takes place. During the larval phase there is an increase in the floating capacity. This is brought about by acquiring strong cilia and increasing the body surface which support these cilia. The ciliary bands running horizontally on the dorsal side and around the bases of the arms develop during the late pluteus stage. Increase in body surface is accomplished by increasing the number of arms. Although there are some variations among different species, most often two more pairs of arms are added. One pair appears between the two antero-lateral arms in front of the mouth (pre-oral arms), and the other pair is formed laterally (postero-dorsal arms).

COELOM AND HYDROCOEL

It was mentioned previously that in the pyramid stage, when archenteron and stomodaeum become connected, the secondary mesenchyme cells shift their position and form two lateral masses on the archenteric tip. These two masses develop into two slender tubes running along the oesophagus and stomach; these are the coelomic vesicles, the rudiments of the future coelom and hydrocoel. Soon each slender tube acquires a constriction in the middle separating it into anterior and posterior chambers. In the meantime, the anterior chamber of the left coelom constricts off a new chamber in an obliquely posterior direction. This is the hydrocoel which will give rise to the adult water vascular system. However, the hydrocoel and the left anterior chamber remain connected by a communicating tube, the stone canal and a swelling at the junction between the anterior chamber and the stone canal is the rudiment of the axial sinus. Although the anterior chamber of the right coelomic vesicle likewise constricts off a small chamber a little later than the left side the communication between the two is severed, and the small chamber becomes isolated and is eventually pushed to the dorsal side of the adult to become the madreporic vesicle.

In addition to the stone canal and the hydrocoel, the anterior chamber of the left coelom sends out toward the dorsal side of the larva another tube which opens to the outside. Its opening constitutes the madrepore or dorsal pore and the tube leading to it is the pore canal. This pore canal is found widely among Echinodermata but its presence is believed to indicate a kinship between the Echinodermata and Hemichordata (Fell, 1948).

Metamorphosis: Echinus Rudiment

Preparation for metamorphosis begins at the body surface of the pluteus. A shallow depression appears in the ectoderm between the left postero-dorsal arm and the post oral-arm of the same side exactly above the hydrocoel. This depression is the amniotic invagination which later expands toward the inside like a sac and comes into contact with the hydrocoel.

The hydrocoel in the beginning, as seen directly from above, appears as a circular structure but due to extension at two places becomes crescentic. Later the two horns of the crescent bend inward as they elongate, finally fusing by their tips to form a complete ring. The amniotic invagination settles onto this ring, in the meantime closing off its opening and sinking below the surface. This cavity is the amniotic cavity and its thin ectodermal ceiling is called the amnion. The bottom of the cavity is made up of columnar ectoderm cells which lie directly over the hydrocoel (Fig. 9.9 b Kume). The amniotic cavity and the hydrocoel are collectively called the *echinus rudiment*, because this unit is the centre of formation of the adult mouth and its surrounding structures.

The next change is the formation of five outgrowths from the ring of the hydrocoel, extending obliquely upward and converging toward the centre. Since such extensions of the hydrocoel push up the bottom of the amniotic cavity, they project into the cavity as five processes. These are the rudiments of five tube feet, representing the tips of the five ambulacral zones of the adult. In the adult, the tube feet found in the ambulacral zones are usually paired, but the first tube feet formed in the larva are unpaired ones which lie at the very tips of the radial canals. Their presence is a feature common to all echinoderms.

The next change that follows is the formation of five radial ridges alternating with the tube feet on the lower side. The top of each ridge then begins to extend horizontally toward the sides like a pair of fins and the fins of adjacent ridges unite so that it is as if the floor of the cavity is covered by another layer. Between the floor and the second layer is the epineural space which will contain the nerve ring. During this time the mesodermal cells derived from the posterior coelomic chamber of the left side migrate into the five radial ridges. These elements constitute the perihaemal system, the main role of which is to form the haemal system of the body. At the centre of the floor of the amniotic cavity the ridges converge and the oral field is eventually formed and also the teeth.

A glance at the adult anatomy shows that immediately beneath the outer covering of epidermis, there is the nerve ring next the teeth and under these the ambulacral ring. From this it can be seen that practically all the important structures of the adult mouth region are already present within the echinus rudiment. A little later, spines make their appearance on the side wall of the amniotic cavity. On the body surface of the advanced pluteus, spines and pedicellariae and a part of the adult test are formed. The spines which are found in the amniotic cavity have pointed ends, while those appearing on the larval surface area square rods with flat ends.

METAMORPHOSIS

When the adult organs are formed as mentioned above, it only remains to break open the roof of the amniotic cavity and expose the structures within it. The process of sea urchin metamorphosis consists simply in bringing the echinus rudiment to light by the rupture of the amnion. The entire change takes only a few hours. The amount of epidermis making up the inner surface of the amniotic cavity is obviously insufficient to cover the entire larval body after inversion, so that enormous expansion of this layer is necessary. Together with this expansion, the five branches of the ambulacral ring reaching into the tube feet are also greatly extended. As these branches elongate, the azygous tube feet are always pushed ahead at the distal ends of the branches, while behind them paired tube feet are laid down to form the radial ambulacral zones of the adult. When a sea urchin reaches the completely mature adult stage, the azygous tube feet are found to have been pushed all the way around to the dorsal side. With the eversion of the amniotic cavity, the arms of the pluteus are immediately resorbed; the skeletal rods, which are absorbed only slowly, are left naked on the body surface until they break off and are discarded, enabling the young sea urchin gradually to round up. At this point the body axis again rotates through 90° to bring the left side of the animal down to the substratum: it then walks away on its five tube feet. The perforation of the mouth and anus take place at a still later time. The strongest feature in this whole metamorphosis of the sea urchin is the fact that the left half of the pluteus body is a solo player which monopolizes the stage.

Metamorphosis of Clypeastroidea

In the development of this group many observant features are found, because of its exceptionally fast rate of development. The pluteus stage is reached on the day following fertilization, the pluteus has only one pair of post-oral arms and the larval mouth never opens. The amniotic invagination appears on the median plane in the part where a mouth would usually be formed; this determines the median position of the echinus rudiment between the arms, and may have something to do with the failure of the mouth to open. Even in this group, the left coelom maintains its dominance. The swimming stage lasts for only one day and memtamorphosis is complete on the third day.

Ophiuroidea

EARLY STAGES

The early cleavage takes place in succession. The 16-cell stage shows no special cleavage patterns like that of the sea urchin. It consists of four tiers, each made up of four equally sized cells. The fertilization membrane dissolves and the blastula comes out. The only difference with sea urchin development is the cells on the animal pole side become vacuolated. The first major deviation from the sea-urchins is in the mode of coelom formation. In the case of *Ophiothrix*, the coelom formation is typically enterocoelic. The tip of the archenteron becomes thin, is pushed out, and separates as an independent sac which later divides into two vesicles. At the same time, a pair of arms are formed by the primary mesenchymal cells. These are supported by triradiate spicules. The first pair of arms of the brittle star larva is not homologus with the first post-oral pair of the pluteus. The first pair in the brittle star turn out to be the postero-lateral arms which appear last in sea-urchins or, often omitted entirely. These larvae eventually acquire four pairs of arms, although they resemble sea urchin pluteus in appearance, they fail to develop pre-oral arms. For these reasons, the larva of the brittle star is called an ophiopluteus and is distinguished from that of the sea-urchin, which is called the echinopluteus. The changes in the internal organization of the larva closely resemble those of sea urchins; the changes that take place are: connection of the archenteric tip to the stomodaeum, formation of tripartite digestive tract, transformation of the blastopore to anus, the separation of the coelomic vesicles into anterior and posterior chambers and the formation of the hydrocoel by the left anterior chamber only. The madrepores and pore canals of both sides develop equally for a short time, but since the right ones soon degenerate, the final result is the same as that in the sea urchin.

METAMORPHOSIS

The growth of the left coelom is the first step in metamorphosis. The posterior chamber of the left side invades the right half in the form of two projections (Fig. 12.1) which fuse to occupy most of the posterior half of the larva. With the enlargement of the left anterior chamber, the left madrepore is pushed to the right as far as the median line, while the hydrocoel extends along the left side of the oesophagus, assuming a crescent shape with five finger like projections (Fig. 12.2). The two horns of the crescent in the ophiuroidea further lengthen into the right half of the body, one on the dorsal side and the other on the ventral so that after the ends of the horn fuse, the comple-

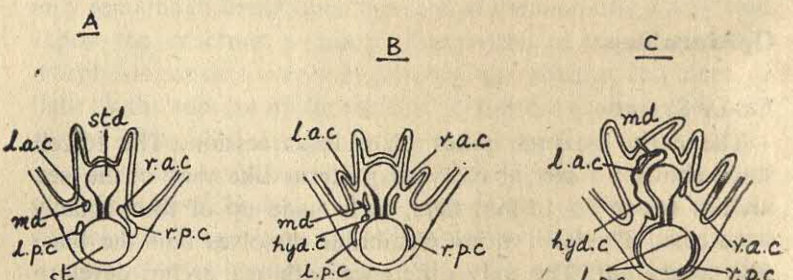

Fig. 12.1. Formation of coelomic system in brittle star. hyd.c—hydrocoel: lac—left anterior coelom lpc—left posterior coelom; md—madreporic canal and its opening; rac.—right anterior coelom; rpc.—right posterior coelom; st—stomach; std—stomodaeum.

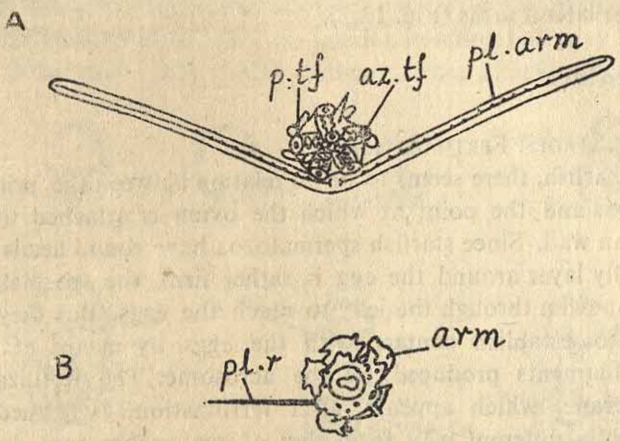

Fig. 12.2. Late ophiopluteus and adult.
a—Ventral view of late ophiopluteus larva.
b—Dorsal view of young brittle star.
arm—adult arm; aztf—azygous tube foot; pl.arm—postero lateral arm; pl.r—posterolateral rod; ptf—zygous tube foot.

ted ring canal encircles the stomodaeum. This expansion of the left hydrocoel causes the median axis of the larva to bend toward the right (Fig. 12.1).

Five chains of mesodermal elements destined to form the haemal system are sent out from the left posterior coelom to alternate with the five lobes of the hydrocoel. Five ectodermal ridges appear along these five chains. The upper parts of these ridges extend horizontally like fins and later fuse to form the epineural space. These changes are practically the same as in sea-urchins. A marked difference is, that in sea-urchins, all organs are formed within the echinus rudiment on the left side of the body, while in the ophiuroidea, the hydrocoel begins on the left side but moves to a median position. The larva loses its arms, except for the postero-lateral pair, a pentagonal adult test is formed between the arms, and the left coelom is pulled into the test by the contraction of the epidermis. The pentagon, which represents the disc of the future brittle star, already appears in this stage as a little star suspended from the pair of arms. By the time five short tube feet begin to protrude from the disc the ophiopluteus loses its capacity to float, sinks to the bottom and becomes an adult by casting the postero-lateral arms (Fig. 12.2).

Asteroidea

EARLY STAGES: FERTILIZATION

In starfish, there seems to be no relation between the primary egg axis and the point at which the ovum is attached to the ovarian wall. Since starfish spermatozoa have round heads and the jelly layer around the egg is rather firm, the spermatozoa cannot swim through the jelly to reach the eggs. But they are able to establish contact with the eggs by means of very long filaments produced by the acrosome. The fertilization membrane, which appears after fertilization, is formed in a slightly different way from that of sea urchin eggs. In sea urchins the presence of vitelline membrane, which is the precurser of the fertilization membrane, can be established, indirectly by experiments but cannot be visually detected. Because of this the impression is created that the *de novo* membrane is formed after the sperm entry. In starfish eggs, the vitelline membrane is visible before fertilization and the rate of its elevation as the fertilization membrane is very slow. It continues to rise until almost the first cleavage. When the first polar body is already formed and lying under the vitelline membrane when the eggs are inseminated, it adheres to the inner surface of the membrane when this rises away the egg surface, and becomes stretched flat as the fertilization membrane expands. If the second polar body is extruded after the membrane has been elevated it is not subjected to this strain, and lies free and spherical in the perivitelline space. A hyaline layer, thinner than those of sea urchin eggs, can also be seen surrouning the egg surface.

MORULA, BLASTULA, GASTRULA STAGES

The first, second and third cleavages are much like those of sea urchin eggs, but in the fourth cleavage, no size differences occur among the 16 blastomeres. As the cleavage proceeds a blastocoel appears at the centre of the embryo and the swimming blastula breaks out of the fertilization membrane as in sea urchins. The starfish blastula proceeds directly to the gastrula stage with no intervening formation of primary mesenchyme cells. While the blastocoel is quite spacious in the gastrula, the archenteron remains relatively small extending half way across the blastocoel (Fig. 12.3). After a time, mesenchymal cells provided with pseudopodia creep out from the tip of the archenteron into the blastocoel. The wall of the archenteric tip then thins and bulges and two vesicles separate from it, one on each side. These are the coelomic vesicles, and with their formation the starfish larva for the first time acquires bilateral symmetry. The next process is the tripartition of the digestive tract. The foremost part of the oesophagus is connected with the stomodaeum, the middle part expands as the stomach, the slender posterior part forms the intestine and the blastopore functions as the anus. All these structures are similar to those of sea urchin larva (Fig. 12.3). At this stage, the coelom exists as two vesicles on the right and left sides of the oesophagus.

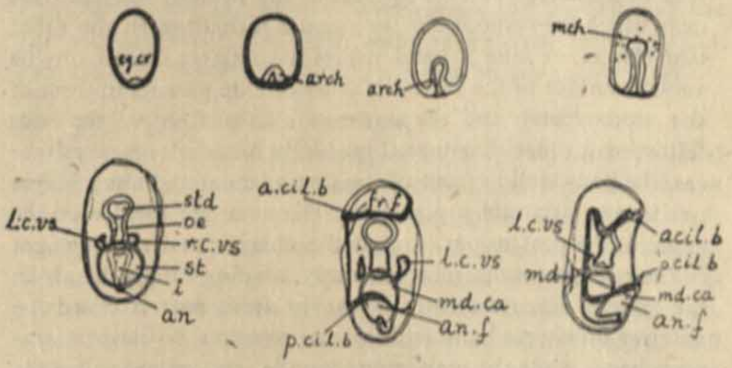

Fig. 12.3. Early development in the starfish, *Asterias*.
a—Blastula.
h—Successive stages of young gastrula.
e—Young larva (dorsal).
f—Ventral view of early bipinnaria larva.
g—Lateral view of same.

a.cil.b—anterior transverse ciliary band; an—anus; anf—anal field; arch—archenteron; fr.f.—frontal field; i—intestine; l.c.vs—left coelomic vesicle; mch—mesenchyme cell; md—madrepore; md-ca—madreporic canal; oe—oesophagus; p.cil.b—posterior transverse ciliary band; r.c.vs—right coelomic vesicle; sg.cv—segmentation cavity; st—stomach; std—stomodaeum.

Bipinnaria: External morphology: The swimming larval stage of the starfish which corresponds to the pluteus of the echinoidea and ophiuroidea has no spicular skeleton and is oval in shape (Fig. 12.3). There seems to be a correlation between this lack of a skeleton and the absence of the primary mesenchyme cells which are responsible for spicule formation in the other two classes. A ciliary band makes a complete circuit on the ventral surface of the larva, its anterior side passing in front of the stomodaeum and the posterior side in front of the anus following a squarish course (Fig. 12.3). As development advances, the body wall in front of the stomodaeum and anus bulges out in two separate protrusions. The cilia are located on the edges of the protrusions, so that the complicated development of their shape results in a sinuously winding ciliary band. In the simple, squarish, early larva, the front part is called the anterior transverse band and the rear part, the posterior transverse band, while the remaining two side sections are called the longitudinal bands (Fig. 12.3). The part of the larval body in front of the anterior transverse band is the frontal field and that behind the posterior transverse band is the anal field. These fields protrude more and more and since the frontal field bends back and the anal field bends forwards, the ciliary band takes the shape of two apposed horse-shoes. The gap between the ends of the horse-shoe surrounding the frontal field becomes narrower, and narrower until it is finally cut off from the rest of the band to make an independent circuit. This double circle of ciliary band is a diagnostic character distinguishing the bipinnaria larva of the starfish from the auricularia of the sea cucumber. The starfish larva proceeds to form several pairs of lateral protrusions, which are named in accordance with the system of nomenclature adopted for the pluteus. Since these protrusions are also rimmed with ciliary band, the overall pattern formed by the band has somewhat the look of a bipinnate leaf, hence the starfish larval name of *bipinnaria*.

Coelom formation in the bipinnaria follows the typical course common to the other members of the Echinodermata. In *Asterias vulgaris* the coelomic vesicles are long tubes paralleling the digestive tract and running almost the whole length of the larva. The left vesicle sends out a pore canal towards the dorsal side near the border between the oesophagus and the stomach and communicates with the outside through the madrepore. The left coelomic vesicle constricts slightly at the junction of the pore canal so that a demarcation of the anterior and posterior chambers can be seen. Immediately the anterior chamber forms the hydrocoel which is crescent shaped with the five projections. The right coelom forms pore canals which open to the outside and these are closed within a short time.

BRACHIOLARIA AND METAMORPHOSIS

At the beginning of metamorphosis, the larva becomes fixed to the substratum. The advanced bipinnaria larva acquires a new projection at the anterior frontal region. This projection is quite prominent and lies between the pre-oral and median dorsal arms. The projection is divided into three branches at its tip and each one of the branches is provided with a fixing disc at the end. This projection includes the anterior coelom and differs from the arms of the bipinnria and hence it is called *brachiolar arm*. The bipinnaria fixes itself to the substratum by this arm in an inverted position. During this sessile period the larva is called as the *brachiolaria*. The brachiolar arm is called the stalk and the rest of the body is known as the disc. The stalk surrounds the anterior coelom and encloses the stomodaeum and the oesophagus. By this time, the right and left anterior and posterior chambers unite mutually so that the entire coelom consists of only two parts, the anterior and posterior.

Sooner or later, the digestive tract becomes a blind sac, by losing connections with stomodaeum and anus. At about this time five ectodermal bulges appear on the right side of the body surface and become arranged in a circle. These are the rudiments of the future arms of the starfish. The crescent-shaped hydrocoel of the left side becomes a complete ring and its five branches get into the five bulges of the opposite side. As the ectodermal pentagon increases in size it comes to enclose the posterior coelom and the stomach beneath it. The tips of the five branches of the hydrocoel develop into azygous tube feet. The blind sac of the digestive tract begins to push out two horns, one to form the future oesophagus and the other, the adult intestine.

With these changes, the lateral arms of the bipinnaria become less and less conspicuous and the stalk also gets reduced in length. As the stalk shrinks, the brachiolaria, while remaining fixed turns its axis by 90° so that the pentagonal test comes around to the posterior side. When the test grows to its full size, the whole body becomes enclosed in it and the hydrocoelic pentagon fits over it like a lid. After the complete absorption of the stalk, its place is taken by the oral surface of the adult starfish.

ABERRANT MODES OF DEVELOPMENT

The inclusion of a sessile stage sets apart the development of the Asteroidea as clearly different from those of the Echinoidea and Ophiuroidea. Even among the starfish two types of cases can be found which have secondarily given up the habit of fixation. One form precociously forms a pentagonal test on the posterior side of the swimming bipinnaria. Such a mode of development closely parallels that of the ophiuroidea. The other type seems to be the result of extreme adaptation, an unusually generous amount of yolk permitting an abbreviation of the developmental process which omits fixation and even feeding. In the species, *Asterina gibbosa* the adult body is immediately formed without any intervening stages. The pre-oral lobe of this animal is quite big and maintains sufficient floating capacity. Among the starfishes, *Solaster* usually has about ten arms. This deviation does not appear in the larval development until after a hydrocoel with the usual five branches has first been formed.

Crinoidea

One crinoid, which has been well investigated is the European species, *Antedon rosacea*. At spawning, the eggs of *A. rosacea* becomes attached to the arms of the mother by a mucous secretion. Such a brooding habit has introduced considerable specializaion into its embryology. The following account of crinoid embryology is based on *Comanthus*, supplemented with information obtained from *Antedon*.

Early development: The gametes are produced within the pinnules which are arranged in two rows on each arm. Since the pinnules have no definite opening, spawning takes place by the rupture of their walls. The eggs begin to undergo maturation a little after noon on the spawning day and are liberated within 3-4 hours. The sperms are released usually before the eggs and are usually accompanied by large quantity of mucus. The fertilization membrane of the crinoids in general is a spiny polygon with a large number of facets. The first cleavage takes place after about an hour. The cleavage furrow is heart-shaped and starts from the animal pole. The second and third cleavage follows the usual pattern resulting in an 8 cell stage like that of the sea urchins. The four cells of the animal pole side seem to be a little smaller. At the morula stage, these animal cells adhere together more compactly, causing the vegetal pole side to remain open for longer time.

SWIMMING LARVA

The eggs hatch out by dissolving the fertilization membrane. They soon develop into the typical crinoid larva commonly called as the *doliolaria* larva. It is provided with an apical tuft and five rings of ciliary bands. The anteriormost band is incomplete ventrally. During the free swimming stage the larva does not feed. While it is swimming, a fixing disc is formed within the anteriormost ciliary ring, and a stomodaeal invagination appears between the second and third rings.

SESSILE STAGE

The larva at first attaches obliquely upside down because of the position of the fixing disc. This however changes within 2-3 days and the posterior part of the body stands perpendicular to the substratum. While this change is taking place, the stomodaeum changes its position from ventral to the posterior side, simultaneously sinking from the surface, so that when the larva stands erect, the stomodaeal invagination forms an independent blind sac buried in the posterior (upper) end of the body. This blind sac is called as the vestibule. Within two to three weeks, the roof of the vestibule bursts open and ten tentacles make their appearance. These tentacles are located at the sides of five oral valves which are situated in the intertidal zones surrounding the mouth. Within four weeks five more azygous tentacles make their appearance particularly on the radia, making the total number to fifteen. The larva at this stage is able to contract and relax these tentacles. In the fifth week the shape of the disc changes and starts giving extentions which will become the future radial arms. At the tip of these arms five azygous tentacles make their appearance. As the arms increase in their length, paired tentacles are added proximal to the azygous ones. By this time, ten other small tentacles can be seen directly encircling the mouth at the inner sides of the oral valves. The larva of this stage is called the *pentacrinoid*.

In the seventh week, the arms continue to elongate, and their tips becomes bifurcated. Although this gives the impression that the number of arms has increased to ten, close examination makes it evident, that the azygous tentacles have been left behind at the points of bifurcation. This fact suggests that the arm proper probably stops at the azygous tentacle, and what appears to be a fork may actually be the point of division of the arm into two side branches. In contrast to the outward shift of the azygous tentacles, the positions of the perioral tentacles do not change. Considering the anatomy of the Echinoderms as a whole, it appears that the water vascular system of the tentacles around the mouth is directly connected to the ring canal, while that of the azygous tentacles connects with the tips of radial canals, which lead off from the ring canal.

After the fifth week, an anus develops at the peripheral side of one of the oral valves, the ectoderm raising up around it to form a prominent papilla. Since the anus is situated in an intertidal zone, it does not affect the symmetry of the arms.

Two significant changes occur in the eighth week. One is, the arms which fill now could only roll inward towards the mouth, suddenly become able to bend outward. The movement of the arms (flexion and extension) constitutes the most common movement of the adult animal. The second change is the appearance of cirri. When they first appear, the cirri are directed upward and in this condition it is quite impossible for them to grasp the bottom. Since the animals have acquired the capacity to bend the arms outward by this time, they cling to the bottom by their arms and begin to creep about within the radius of the stalk-length.

FREE-STAGE

By the tenth week, the two branches of each arm grow longer and produce pinnules which are arranged alternately from the base to the tip. The young animal assumes the colour of the full grown adult and finally cuts itself loose from the stalk.

Concluding Remarks

From the foregoing account it can be noted that in spite of many superficial differences, there is a similarity running through the various classes which can be thought of as either a common basis or a general trend. The phylum Echinodermata is often divided into two groups, the Pelmatozoa, which includes the essentially sessile animals comprising the Crinoidea, and the Eleutherozoa, which are further removed from the sessile habit and includes the four orders, the Echinoidea, Asteroidea, Ophiuroidea and Holothuroidea.

Let us first consider the patterns of the ciliary bands in the larvae of the Echinodermata. As is evident from the figure, the ciliary band patterns can be roughly divided into two types. One type is characterized by five rings of cilia (lowest row) and the other has but one sinuous circuit of cilia (first to fourth horizonal rows). Although the bipinnaria alone has two circles, which show exceptionally complicated windings, it can easily be included in the later type; while *Ophiura brevispina* with its five rings of *cilia,* is an exception among the brittle stars. Holothurian development is interesting because it starts with a single ring in the auricularia larva and ends with five rings in the doliolaria. If the various types of ciliary bands are traced back to the beginning of their formation, the differences among the four classes become slighter and slighter and finally possible to include them in a single common pattern, consisting of a squarish band lying between the frontal and anal fields. If the more advanced stages are grouped, we find the pluteus type on the one hand and the bipinnaria and auricularia type on the other. However, the difference between them is only superficial. In the body of bipinnaria or auricularia, the body remains relatively flat, while in the pluteus it is bent and doubled upon itself toward the ventral side. In other words, protrusion of the frontal and anal fields occurs equally in all these larvae. In the bipinnaria and auricularia this protrusion involves only the body wall, the body axis being left unbent. In the pluteus, however, the stomodaeum and anus are extended together with the surface area, bending the body axis into a V. In order to accommodate its structure to this bending, the pluteus has to turn its axis by 90° from the direction of the gastrula stage.

If the phylum Echinodermata as a whole is regarded as moving along the path from a sessile to a free moving life, among the Eleuthrozoa, the Asteroidea with their brachiolaria stage can be said to be the most conservative. The Asteroidea become free by first moving the structures of the left side toward the stalk and stomodaeum to acquire radial symmetry and then absorbing the stalk. By this shifting of the internal organs, the larva executes an internal revolution of 90°. This invention of the Asteroidea was adopted by the Holothuroidea and Ophiuroidea. The Echinoidea, however, follows a formula which is one step further removed from the tradition of sessile life. The stalk is completely lost and is replaced by the echinus rudiment.

Holothuria

The study of holothurian embryology began in 1880, when Muller discovered the auricularia larva. Our knowledge of holothurian development is scanty in comparison with the information which has been accumulated concerning other classes such as the Echinoidea and Asteroidea.

EARLY DEVELOPMENT

The mature holothurian egg is spherical or rather ovoid. In most cases the eggs are nearly transparent, but there are also some opaque ones. In general, they are larger than those of sea urchins or starfish; ranging in size between 0.2 mm to 3.5 mm. All the eggs are homolecithal. Four types of eggs are found in a ripe ovary; (1) small, irregularly shaped eggs with a prominent germinal vesicle; (2) regularly shaped eggs with a germinal vesicle; (3) regularly shaped eggs without a germinal vesicle; (4) irregularly shaped eggs without a germinal vesicle, which sometimes occur in clusters. Out of these four types, only the third type are fertilizable.

The first and second polar bodies are given off from the animal pole on insemination. Cleavage is total, equal and the arrangement of the blastomeres, in most cases is schematic. In the holothurians, meridional cleavage alternates with horizontal from the third cleavage onward. After 32-cell stage, the time of cleavage becomes different for different blastomeres. The direction of cleavage loses its uniformity and the radial arrangement becomes more or less disordered in many cases, leading to slight size differences among the blastomeres. The cleavage proceeds and by the time the number of blastomeres reaches about 512, the blastula is fully formed and each cell acquires a cilium. At about this time, the vegetal side of the embryo becomes somewhat flattened, and some of the cells from this region are freed into the blastocoel, where they give rise to mesenchyme. After the cilia develop, the blastula begins to rotate within its membrane, and two or three hours later it breaks the membrane and hatches. The direction of rotation is mainly dextral as seen from the animal pole.

The recently hatched larva is nearly oval, about 190 μ in height and 170 μ in width. As it gradually increases in size it grows more in height than in width. Eventually, the vegetal side flattens, an invagination at its centre leads to the formation of an archenteron, and a typical gastrula is formed. The larvae mostly swim about near the surface of the water. The archenteron elongates together with the body and the amount of mesenchyme in the blastocoel increases, but there is no formation of a larval spicular skeleton.

The developmental changes in the archenteron after this stage are different depending on whether or not the larva passes through an auricularia stage. In species having an auricularia stage, the archenteron grows until it reaches about half the body length. Its anterior tip then turns toward a depression which has appeared in the centre of the body wall slightly anterior to the midpoint. The bent part of the archenteron elongates, unites with this stomodaeal invagination and becomes the oesophagus. The straight part of the archenteron, which still lies parallel to the body axis, enlarges and differentiates into a stomach and a long, slender intestine. The blastopore persists while the intestine is being developed and changes into an anus, and its opening gradually shifts round toward the side where the mouth is forming. These changes practically complete the development of the digestive tract.

The internal changes that take place are more complicated. Cells proliferate within the body cavity near the turning of the archenteron. Some of these cells extend to form a tube towards the mid-dorsal line, whereas a few form a pouch near the base of the oesophagus. The tubular part gives rise to an opening on the dorsal surface, the *dorsal pore* while the intervening tube becomes the *pore canal*. The rest of the structure increases in size and differentiates into three regions, one running along the anterior dorsal side of the stomach, another spreading laterally near the anterior part of the stomach and a third extending longitudinally parallel to its lateral surface. These are called the anterior, central and posterior enterocoel. Since these will later develop into the body cavity and hydrocoel, they are given the collective name of *enterohydrocoel* before such differentiation takes place.

Externally the body continues to elongate, and becomes angular at the vegetal pole side and rounded anteriorly. The cilia which were covering the whole surface disappear and only those on marginal ridges remain giving the characteristic shape of the larva. The cilia are borne on a single, continuous band which is slightly thicker than the rest of the body wall, and curves to form the ring shaped borders around the anterior and posterior ends of the larva.

In forms which do not pass through the auricularia, the gastrula stage is externally almost identical with that described above, but the internal development includes a conspicuously different stage. This stage involves a striking change in the archenteron and a rapid proliferation of mesenchyme cells. At the beginning, the archenteron elongates and its anterior end turns slightly towards the left ventral side, and at the same time a separate pouch-like structure is formed. This is the entero-hydrocoel, which continues to grow for a while in connection with the archenteron, but eventually separate from it.

After the archenteron grows to a length, equal to about half that of the body, it turns ventrally and elongates to make contact with the stomodaeal invagination which has developed in the centre of the ventral surface. As soon as these two structures come together, a mouth opening is formed. The blastopore remains in its original position at the posterior end of the larva and forms the anus: The part of the archenteron which is continuous with the oesophagus widens and becomes the stomach. The entero-hydrocoel, which is shorter than the archenteron, differentiates into a wide hydrocoel and an enterocoel. The connection between these two is lost and the hydrocoel gives rise to a slender rudiment of the pore canal which extends to the mid-dorsal line, as well as to three bulges which are the rudiments of tentacles. The enterocoel divides into left and right parts which grow downward around the archenteron. This larva, eventhough, externally looks like a gastrula, is advanced in its internal differentiation to the later part of the auricularia stage.

LARVAL STAGE: AURICULARIA

This larva resemble the bipinnaria larva of the Asteroidea but can be distinguished from them by two characteristics: (1) the ciliary band eventhough complicated, is continuous, (2) the processes extending from the peripheral ridges are always short. The ciliated band forms a small, circular pre-oral loop above the mouth and another small anal loop anterior to the anal opening. The processes hardly develop at all. This stage is reached in about 45-46 hours. From this time the larva begins to feed and the rate of growth depends on the suitability of the food.

As time passes, the auricularia larva increases in size and its ciliated band becomes more convoluted. The winding of the ciliary band is bilaterally symmetrical. The projections are much shorter than those of sea urchin or starfish larva, but they are given the same names. There are six pairs of processes; the preoral arms near the mouth, the post-anal arms near the anus, the postero-lateral arms at the rear end of the body. The antero-dorsal, mid-dorsal and postero-dorsal arms are on the dorsal side. Calcareous larval spicules appear for the first time several days after the beginning of the auricularia stage. In most cases one spicule is formed at the tip of each postero-lateral arm.

HYDROCOEL AND BODY CAVITY

The hydrocoel and the enterocoel which are separated from the enterohydrocoel at the end of the gastrula stage, grow and undergo changes in form, while the digestive tract simply increases in size. The hydrocoel lies at the left side of the oesophagus and bends upward to surround it dorsally. The water vascular system of the tentacles and the rudiment of the ring canal are formed as large and small vesicles respectively. By the end of the larval stage, these vesicles give rise to the primary tentacles and polian vesicle. As a result of this development, the larva loses its bilateral symmetry internally, while retaining it externally. The pore canal extends to the mid-dorsal line.

The enterocoel forms only on the left side, but one or two days later another vesicle separates off on the right side. The left enterocoel extends from the anterior side of the stomach, and the right enterocoel from near its middle. Both grow downward towards the stomach. The mouth is a large, mushroom shaped opening boundered by cilia. Towards the right and left sides of the mouth, in the depressed oral field, slender, bent, raised lines, called as larval nerves can be seen. As the larva grows, a branch extends outward from the point of bending, forming a Y-shaped structure.

METAMORPHOSIS

Holothurian metamorphosis from the auricularia stage involves a severing and rearrangement of the ciliated band. The larva changes its form and gives rise to a barrel shaped *doliolaria* or pupa stage, encircled by loop like rings of cilia. As the result of metamorphosis the larval length becomes abruptly reduced. The disappearance and recombination of parts of the ciliated band are due to unequal growth of the epidermis. In the pre-oral loop, a central section and two lateral sections persist, and three similar sections represent the anal loop. At the sides, there are three fragments of the ciliated band from the antero-dorsal, mid-dorsal and postero-dorsal processes, one from the mid-dorsal depression between the mid-dorsal and postero-dorsal processes, making a total of four. These sections join together to form five ciliated rings, each united with other sections. The first ring is formed of sections of ciliated band from the oral region and the left side of the pre-oral loop. Two sections from the antero-dorsal process and one from the right side of the pre-oral loop form the second ring. The third ring consists of pieces of the band from the mid-dorsal process. The fourth ring is made up of two fragments from the postero-dorsal process and two from the sides of the anal loop. The fifth ring is formed by the anal loop. The fifth ring is formed by the union of fragments from the two postero-lateral process.

In the holothurian species which do not pass through an auricularia stage, the cilia, which have been covering the whole surface of the gastrula, begin to disappear from parts of the ventral side, and this process continues toward the dorsal side until several ciliary rings are formed. Generally, four or five ciliary rings are seen.

As a result of metamorphosis, the mouth, which was in about the centre of the ventral surface during the auricularia stage, shifts to the anterior end, and the anus moves to the posterior end. While these external changes are going on, the internal organs are also developing. The hydrocoel grows around the oesophagus and finally its left and right sides unite ventrally to form the circular ring canal, which accompanies the mouth as it moves anteriorly. At the same time the larval or primary tentacles elongate and the polian vesicle increases in size. The ring canal gives rise to the radial canal which extend between the five tentacles and elongate posteriorly in the opposite direction from the tentacles. Between these, the pore canal extends to the midline in the centre of the dorsal ridge, and its position determines the position of the tentacles, Polian vesicles and radial canals.

The pore canal becomes reduced, the dorsal hydropore is drawn inside the body and by the end of this stage it becomes calcified gradually to form the *stone canal*. This stone canal makes connection with the mid-dorsal point of the ring canal. The radial canals elongate along the body wall. In the holothurians where tube feet are present, the mid-vental radial canal develops faster and to a greater extent than the others and bifurcates distally. At the same time two small pores appear in the posterior ventral body wall and the branches of the radial canal grows toward these, eventually to form the primary tube feet.

The enterocoel extends posteriorly on the ventral side, its right and left sides surround the stomach and intestine and meet with each other dorsally as well as ventrally. The dorsal plane of union coincides practically with the longitudinal body axis, but the ventral plane is diagonal, since the left enterocoel has developed faster in the posterior part while the right enterocoel is larger anteriorly. The membrane formed by the union of the two enterocoels persists in the adult as the mesentery.

The mouth which is surrounded by tentacles becomes protuberant. The stomach and the oesophagus shorten when the body length is reduced, whereas the intestine grows longer and begins to bend to some extent. The anus opens at the posterior end of the body. In the sea cucumbers which have the spherical structures described above, these lie at the sides of the body in close connection with the ciliary rings. They elongate into an oval shape and eventually nearly come in contact with each other. The nerves arise at the bases of the tentacles on the inner face of the ring canal. They elongate along the anterior side of the radial canals, and finally form a nerve ring and five radial nerves. X-shaped calcareous spicules (ossicles) begin to be formed in the body wall. At about the same time calcareous spicules of the same shape appear in the bases of the tentacles. The former are the endoskeletal spicules, while the latter are rudiments of the calcareous ring.

At the end of this stage, the tentacles become covered with ectodermal tissue and parts of them can be seen from the outside, through the mouth opening. The vestibule, surrounding the mouth becomes smaller. During the earlier part of the doliolaria stage, the larva swims vigorously, but as the end of this period approaches, their ciliary movement becomes sluggish and they begin the change toward a more sedentary way of life. Within a short time, the rings of cilia disappear and the young animal becomes completely bottom dwelling. The vestibular invagination is still more reduced so that the mouth is shifted almost to the anterior end of the body, and the five primary tentacles move freely in and out of it. About ten days are required for the young holothurian to pass through a series of gradual changes before it finally achieved an appearance like that of the adult. As the number of X-shaped spicules increase, each spicule also extends its branches and joins with the branches of other spicules to form fenestral plates. These spicules cover the entire body surface.

In the holothurian orders which form tube feet (Podia), the mid-ventral radial canal grows more rapidly than the others. This radial canal divides at its tip into two podial canals. They continue to grow and finally protrude to the outside of the body through two pores formed in the posterior-ventral body wall.

By this time a coelom is formed by the union of the right and left parts of the enterocoel. The coelom is tubular, uniform in diameter and extends from the mouth at the anterior end to the posterior end of the body. At the posterior end it turns back on the left side and runs anteriorly for some distance before turning again posteriorly to connect with the anus. The water vascular system consists of the five primary tentacles, the ring canal and the stone canal. The nervous system is made up of the nerve ring and five radial nerves. The nerve ring surrounds the oesophagus at the bases of the primary tentacles and the radial nerves run along the outer side of the radial canals.

JUVENILE HOLOTHURIAN

The young holothurian increases in body size accompanied by an active formation of the characteristic spicules. There is also an increase in the number of secondary tentacles and tube feet. The colour of the body wall is whitish and somewhat opaque. The oral region opens widely and a mesentery connects the five primary tentacles. Spicules are formed in the tentacles, tube feet, as well as in the body wall. Except for the increased size and thickness, the adult spicules are practically the same as those of the juvenile. The anal teeth grow and surround the anus, and the five large spicular plates between the primary tentacles becomes still larger, acquiring the shape of shields. These project to the outside, covering and protecting the oral region when the tentacles are contracted.

The system of radial canals includes some which give off two tentacular canals, others which have only one and some with none. The secondary tentacles usually originate from radial canals lacking primary tentacles. In the holothurians having tube feet, these increase in number, the position of the newly added podia differing in different genera and species.

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